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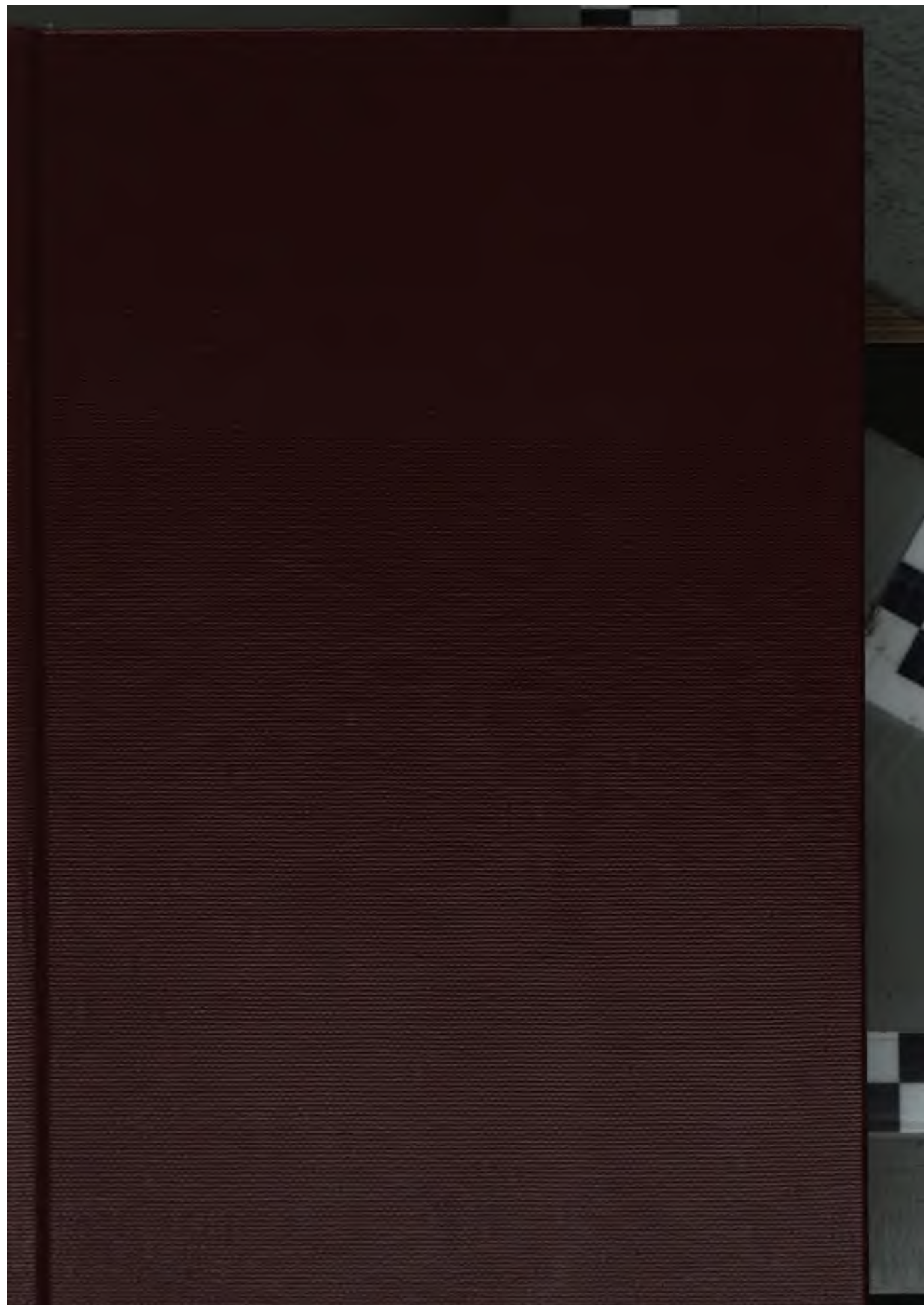
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JOURNAL OF PATHOLOGY
AND BACTERIOLOGY.

**JOURNAL OF PATHOLOGY
AND BACTERIOLOGY.**



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THE
JOURNAL OF PATHOLOGY
AND
BACTERIOLOGY.

*EDITED WITH THE COLLABORATION OF DISTINGUISHED BRITISH
AND FOREIGN PATHOLOGISTS*

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**JOURNAL OF PATHOLOGY
AND BACTERIOLOGY.**

ATHEROMA.

By W. AINSLIE HOLLIS, M.D. (Cantab.) F.R.C.P. (Lond.), *Physician
to the Sussex County Hospital.*

(PLATES I. AND II.)

THE generally accepted view that atheroma is a senile degeneration of the arterial coats, bequeathed in many cases as a legacy to old age by youthful indiscretion, is necessarily both incomplete and unsatisfactory. It is incomplete, in so far as it either overlooks or discredits the many cases of atheroma which occur in the young and the innocent alike. It is unsatisfactory, because it attempts to gloss over our ignorance of the essence of this disease by a term which explains little and implies much. Yet it has been known, on the one hand, for nearly half a century, since Sir James Paget drew attention to the subject, that sclerotic valves were frequently attacked with acute disease, an observation subsequently confirmed and extended by Drs. Goodhart, Osler, and others; on the other, for a somewhat shorter period, through the researches of Professor Virchow, that endocardial and endarterial atheroma were practically identical. My researches into the histology of this protean disease, whether met with in the heart or in the arteries, in an acute or in a chronic form, trend in the same direction, and I feel assured that we shall most truly appreciate the importance of atheroma, as a pathological event, by adopting a comprehensive view of its nature and significance.

THE ETIOLOGY OF ATHEROMA.

The mesoblastic origin of the blood vascular system, and its early seclusion in embryonic mammalian life, from direct communication with the environment, point to nature exercising a watchful care to prevent the invasion of the blood by foreign particles. There are numberless natural safeguards, partly of epiblastic and partly of hypoblastic descent, whose function it is to prevent so grave a mishap. As long as these safeguards remain structurally sound, they meet the requirement of the case admirably. When, however, from any cause, some of them

become unsound, to just that extent the blood is exposed to contamination. The discovery of various microbes in the sprouting growths of infective endocarditis gives presumptive evidence of the presence of foreign particles within the living blood vessels.¹ In the more chronic forms of atheroma, the existence of these organisms within the vessels is mostly unproven, although the researches of bacteriologists, by yearly adding to the list of diseases due to the presence of microbes in the vascular system,² make it clear that few adults can hope to have escaped one or many successful invasions of their blood by these living particles alone. It is, therefore, by no means improbable that advanced atheroma, when found, will have a clinical history pointing to some previous blood contamination by minute intruders. This topic will next demand a few words.

Throughout life the physiological relationship of the hypoblast and the mesoblast is very close, much closer indeed than is that probably existing between the descendants of the epiblast and the latter. In the adult, for instance, the dependence of the body, as a receptive machine, upon the functional soundness of the mucous membrane and its glands is well known. We daily absorb and assimilate many ounces of nutriment. Much of this finds its way into the blood. In health, glandular and other devices, doubtless, render this constant influx of extraneous material for the most part aseptic and so far innocuous. The pulmonary vesicles, again, bring the environment and its minute inhabitants into still closer contact with the blood than even the mucous lining of the alimentary canal does, and yet no evil necessarily results. When however, by disease or injury, an unguarded communication has been established between the blood vessels and the outer world, this aseptic condition of the blood may at any time cease, and indications of atheroma of the vascular walls may appear. In all these cases the epithelium undoubtedly is the chief safeguard against the intrusion of foreign particles into the blood current. In health the epithelial lining of the hypoblast is probably shed frequently, and along with its scales any adherent particles are also removed. This physiological operation usually answers well. When, however, an accumulation of extraneous particles has occurred in some mucous fold or recess, whence removal is

¹ Winge, of Christiania, in the year 1869, appears to have been the first to suggest the presence of micro-organisms in the blood, as a cause of disease.

² I may here enumerate a few of the micro-organisms discovered in the blood by different observers. Pollender described the occurrence of *Bacillus anthracis* in the blood of persons suffering from wool-sorters' disease. It is usually associated with some leucocytosis. Obermeyer, in 1874, first drew attention to the presence of a spirillum in relapsing fever. It is found in the blood only during the paroxysms of the disease. Tubercle bacilli (Meisels), *B. typhosus* (Rütimeyer), bacillus of glanders (Löffler), *B. hydrophobæ* (Bareggi), *Plasmodium malarie* (Marchiafava), and several varieties of streptococci (excluding animal parasites, such as distomata, filarie, etc., which are scarcely micro-organisms), are all of them occasional occupants of the blood vessels. Many others have been noted as occurring in the blood by various observers, but their investigations for the most part require confirmation.

difficult or tedious, these scales of shed epithelium may rest awhile with the other particles, and together with them form a source of irritation to the adjacent tissues, leading to the rapid production of successive layers of mucous scales. If the block is not serious, this cell proliferation is doubtless sufficient to re-establish the functional soundness of the membrane. It may often happen, however, that the rapid cell formation may increase the mischief which it in some cases can avert. And when the intruding particles are living, and capable of spontaneous increase, this failure must be the rule. When, moreover, the intruders are endowed with movement, and are actively hostile to the host, his misfortune is their opportunity. If sufficiently minute they may pass through the so-called cement substance between the epithelial scales into the tissues beyond,¹ or they may find their way there through the denuded basement membrane. The horny epithelial layer, as far as my experience serves me, is rarely penetrated by microbes, so long as it is sound; the epithelial-loving parasites, as described by Foà, Ruffer, and others, may be exceptional. Furthermore, the intruding particles occasionally consist of finely divided inorganic matter. If the observations of C. Isnard on the causation of arterio-sclerosis are confirmed, we shall find such a substance in lead and its salts, many of which are insoluble. Having in some manner eventually effected an entrance into the mesoblastic tissues, and hence by accident or design into the blood, I shall for awhile leave the intruding particles in order to investigate the clinical histories of the cases appended in the table.

Statistics to be of value in atheroma ought to be both voluminous and exhaustive. I cannot hope that the tabulated cases will settle conclusively the etiology, or indeed any other point in the history of this widely distributed disease. My reason for here giving them is that, with two or three exceptions, they represent cases which have come under my own observation. I shall, however, draw attention to a few significant facts, which a perusal of the table elicits. First, there is an exceptional preponderance of males over females in those affected by the disease. There is, again, a considerable number of cases in which a history of one or more attacks of arthritic rheumatism was obtained. This coincides with Isnard's observation. Tubercular disease, with associated pulmonary or intestinal lesions, accounts for several deaths. Malignant disease, including a case of lymphadenoma, is noted in 6 patients. In two of these cases, however, the aorta was converted for a great portion of its length into a rigid calcareous tube, a condition unrecorded in the other tabulated cases. Syphilis is mentioned in three cases only, in one of which it was widely spread. A history of this disease is often very difficult to elicit from a patient. After giving due weight to this circumstance, I still think it doubtful whether

¹ Some writers deny the existence of "cement substance." Heidenhain has attributed considerable mobility to the hyaline adherent borders of the cells lining the small intestine in vertebrates; Hardy and M'Dougall in daphnia; Greenwood in lumbricus.

syphilis exercises any unusual influence in the production of atheroma. If we classify the lesions of the alimentary tract associated with atheroma in the table, we shall find seven cases of ulceration of the stomach, small intestine, and appendix. In two other cases impacted gall-stones are noted. Large abscesses were found in three other patients. The lungs were involved in many cases; the kidneys in a still larger number. I shall return to this subject at a future period. In some cases clinical history points with no uncertain indications to a flaw in the hypoblastic tissues as the portal for the admission of noxious particles into the mesoblast; in many others, for various reasons, it fails to give this evidence.

The earliest age at which I have found distinct indications of atheroma was in the aorta of a girl of $2\frac{1}{2}$ years. In the table there are 12 persons, who were under the age of 26 years at the time of death. These numbers give a percentage of 23 on the cases here tabulated.

The tabulated cases were, with few exceptions, obtained from the *medical* wards of a hospital. Owing to this fact, flaws of the integument due to accident or injury (with one exception, where death was due to a severe burn) are excluded from them. The mammalian integument with its associated glands and horny epidermis, when these exist, constitutes a great defensive and excretory system, by protecting the blood vessels, on the one hand, from unseen intruders, and by assisting, on the other, the kidneys and other excretory organs to remove effete material from the circulation. In performing these functions, it is seldom necessary for the skin to assume the rôle of an absorbent system also; and consequently blood contamination through these channels is probably rare. When, however, as the result of accident or injury to the epidermis, a direct communication is established between the mesoblastic tissues and their environment, the introduction of foreign particles into the blood is only a work of time.

THE DEVELOPMENT OF ATHEROMA.

Although atheroma is pathologically a general disease, inasmuch as it may occur at different and distant points of the vascular system simultaneously, or within a short interval of time, yet as regards its morbid anatomy it is distinctly a local disorder. Each atheromatous patch has an individual history attached to it, which includes its development, its growth, and, in some instances, perhaps, its decay.¹ The patches, again, have this peculiarity; they may grow independently, for they are often found at different stages of growth in the same vessel. Apparently, too, atheroma may spread either by peripheral

¹ As regards the time required for the development of an atheromatous patch, little is known. I have found elevated patches of milky atheroma in the aorta of a boy, aged 11, after a fortnight's illness from appendicitis; in that of a girl, aged 17, after an attack of pneumonia lasting the same period; and in that of a girl, aged 18, 10 days after a severe burn.

extension from a single centre, or it may be due to the coalescence of several diseased foci. In its development and growth, atheroma has a remarkable predilection for certain vascular sites. In a majority of cases this selective tendency of the disease is readily followed, although after severe and protracted illness the extent and variety of the morbid changes may render the process difficult. I now propose to enumerate, so far as my observations will permit this course, the chief localities atheroma affects in its development and early growth.

Possibly atheroma springs most frequently from the endothelium at the commencement of the aorta. If we take the vascular ring, including the aortic cusps, the sinuses of Valsalva, the orifices of the coronary arteries, and about 2 inches of the surface of the vessel beyond the attachment of the valve, we shall retain the chief structures commonly affected with this disease in its various phases. The mitral valve and its appendages, the chordæ tendineæ, are also very frequently attacked, especially with soft fringe-like growths. The endocardium, the pulmonary and tricuspid valves, the remainder of the aorta (especially the upper part of the great sinus), the lesser curvature of the arch, the bifurcation, and the orifices of the large branches, the circle of Willis, and the renal arterioles are more or less often the seat of the disease. Probably no portion of the vascular system is exempt from the ravages of atheroma.

There are certain structural peculiarities of the sites in the aorta and elsewhere most commonly affected by atheroma. My experience is that the aortic sinuses of Valsalva are of all structures the oftenest attacked; and of them the ridge bounding the upper edge of each appears to be *par excellence* the starting-point of atheroma. In this locality, also, the sigmoid lines of attachment of the cusps to the aorta are frequently affected; as are the points of attachment of adjacent cusps, especially that of the attachment of the anterior and the left posterior, and of the two posterior aortic cusps. These remarks apply in a modified degree to the pulmonary Valsalvan sinuses when they are attacked. Upon the semilunar valves themselves, a spot just below a corpus arantii, and thence along a line bordering the lunule on either side, is a favourite situation for atheromatous thickening to commence. The ventricular aspect of the mitral curtains, especially near the points of insertion of the chordæ tendineæ, and the "lines of contact,"¹ just within the free edges of the mitral flaps, are other common sites.

There are some minor peculiarities of structure in the aorta and elsewhere, which seem to influence the deposition of atheroma, and therefore require a passing notice. At the point of attachment of the right posterior aortic cusp in front there arises a broad band of elastic fibres, which, passing upwards along the greater curvature of the arch, originates a series of smaller fibres. These, spreading outwards like the branches of a weeping willow, sweep in bold outlines to the under

¹ To quote the words of the late Dr. Sibson.

surface of the arch, where they meet another band of fibres on the floor of the vessel. The transverse and longitudinal ridges, so formed in the intima of this portion of the aorta, are the favourite situations for atheromatous dépôts. Again, if the endothelium of an artery is examined closely, it will be found to be scored by numberless fine lines, each representing a depression in the arterial intima, and functionally no doubt corresponding to a "line of least resistance" on the skin of our palms and soles. Atheroma mostly commences upon and follows awhile the little ridges of endothelium between these lines.¹ On the boundary ridge of the sinuses of Valsalva, for example, these lines are mostly at right angles to the ridge, and atheroma appears in the first instance as a series of whitish striæ on a creamy background. (Plate I. Fig. 10 shows to some extent this peculiarity of growth.) Finally, I shall draw attention to the variety of atheromatous growth, frequently observed upon the valve of the heart, very rarely elsewhere. I refer to sprouting growths. These excrescences are, to a great extent, confined to the ventricular aspect of the aortic valve and to the auricular surface of the mitral. At all events these two sites are most usually attacked. Some pathologists consider that the localisation of these vegetations is in great part determined by pressure and tension. This explanation, although plausible, is in many respects unsatisfactory. For we can with difficulty imagine that the tensile stress—to use a phrase well-known to engineers—of a small spot on a sigmoid cusp, or at "the line of contact" of a mitral curtain, differs greatly from that of the adjacent endocardium, which is rarely affected in this manner. In considering the development of the milky plashes of atheroma, found so frequently in the aorta, I have drawn attention to some structural peculiarities which appear to influence the early deposition of this disease upon the endarterium. This structural susceptibility of certain parts of the vascular apparatus to the reception of a virus is, I believe, the chief determining factor in cases of sprouting valvular growths. The late Dr. Sibson pointed to the border of small bead-like cells just within the edge of the flap, as the seat of early vegetative growth in the mitral valve. My own observations upon the aortic valvular growths lead me to expect the primary atheromatous dépôt in a majority of cases on the arterial surface of the cusp between the fibrous ridges below the corpus arantii, whence the disease spreads inwards to the endocardium. This subject will, however, be dealt with more fully at another time. Some sigmoid cusps have a loose fold of endocardium at the edge of the lunule as it passes into the corpus arantii. This flap, when it exists, is often the seat of atheromatous changes. (Plate I. Fig. 3 shows this fibrous flap upon an aortic cusp. Plate II. Fig. 7 is a section through a commencing growth on the cusp of a girl of 8.)

In the preceding pages I have drawn attention to the development

¹ The analogy between the development of atheroma and that of xanthoma (especially when the latter affects the flexures of the joints) is marked.

of atheroma, preferably in certain vascular sites, and to certain structural peculiarities in connection therewith. If this statement of the case is haply the correct one, it may be advisable to inquire how these structural peculiarities react on the blood as it circulates past them, and whether the development of the disease can at any time be ascribed to these causes.

A review of the sites commonly affected by atheroma will show that the favourite localities for outbreaks of this disease are conspicuous for some structural irregularities not generally observable. As an illustration of my meaning, I shall mention the frequency with which the great aortic sinuses are attacked, on the one hand; and, on the other, the bosses and ridges upon the cardiac valves and elsewhere. To take an example, the position of a Valsalvan sinus in its relations to the blood stream is unlike that of any other structure of the body. It is distended at each diastole by a backward thrust of the column of blood in the aorta, or in the pulmonary artery, as the case may be, on the closure of the sigmoid flaps. During the cardiac systole, on the other hand, the pocket is shut off from the blood stream.¹ The surface must, therefore, be alternately swept by tumultuous eddies, as the blood closes the semilunar valves, or bathed in a layer of comparatively quiescent blood. Let me next take the case of a corpus arantii at the aortic orifice. Here we have a distinct obstruction to the blood current, just where the bed is narrowest and the stream is consequently fastest. Atheroma, in my experience, most usually attacks that part of the cusp (in the closed valve), immediately external to the projecting body (Plate I. Figs. 1-4). Now it is exactly at this spot that we might rightly expect a blood eddy once during each cardiac cycle. Again, there are certain portions of the arterial system, where dragging or pulling stresses must modify locally the effects of the circulation upon its elastic walls. I allude especially to the lines of attachment of the semilunar valves, and to the orifices of the smaller arteries; localities often affected with atheroma.²

We are so accustomed to consider the blood as a liquid physiological unity, containing in health a definite percentage of semi-solid material, intimately commingled within it, that we too often overlook the wonderful precision with which the relative proportions of these constituents remain practically constant, and the means whereby this end is accomplished. Among the physical processes for ensuring the intimate admixture of the corpuscular elements with the blood plasma doubtless the numerous intravascular eddies and back-currents play their part. However this may be, we find "a little over five millions of corpuscles

¹ According to Brücke, the sigmoid flaps are closely applied to the arterial walls during cardiac systole; Ceradini and others maintain that they float in an intermediate position. It matters not as regards the present contention which of these views we accept. Probably both are partially correct; Brücke explains what happens at the beginning of the systole, Ceradini the position of the valves subsequently.

² Mr. Holmes says: "The aorta, popliteal, and axillary artery seem most liable to disease as being most constantly subject to stretching, and the latter to forcible rupture."

in each cubic millimetre" of our blood, and that these bodies are in the proportion of "one white corpuscle to 600-1200 red ones." Doubtless the endless physiological attributes of the blood are in great part due to the constant numerical relationship that exists between its chief component factors.

I have already pointed out some of the bye-paths, whereby foreign adventitious particles can gain access to the circulation. I shall now endeavour to follow these intruding particles in their wanderings through the blood vessels. When foreign particles find their way into the blood, as, for instance, *Streptococcus pyogenes*, there is no evidence to show that they are at any time distributed through the plasma with a uniformity comparable to that observed by its proper corpuscles. Indeed, pathology points to their dissemination by the blood in scattered groups, a mode of dispersal which accounts, it may be, for the simultaneous appearance, as in the example cited below, of multiple abscesses in distant parts of the vascular system, but which is far removed from simulating the intimate admixture of fluid and solid elements which exist in the blood itself. (Cf. Case 50 of Table.) From what is known of the behaviour of viscous fluids we may assume that the density, the size, and possibly the surface tension of these semi-solid suspended corpuscles are concerned with other conditions in producing the homogeneity of the admixture called blood. The chances, then, of any foreign particles fulfilling the physical conditions necessary to their uniform dispersal through the plasma are extremely small, and we may reasonably refuse to accept them.

Owing to anatomical details I need not recapitulate, the group of intruding particles generally enters the blood vessels by one of the arterioles, capillaries, or venules in the periphery. The chief exception to this course is in the case of intruders directly entering the great central veins by way of the thoracic duct. In the other cases the living microbes or other minute particles quickly find themselves within the veins and hurried along by the steady "viscous flow" of the blood in those vessels. From what is known of the behaviour of viscous fluids in slow motion, notably from Mr. Trouton's clever experiments, we may assume that they are borne past the many valves met with *en route*, without encountering the swirls and whirls to be expected in streams of less viscosity. Neither is there any pretext for hindrance or delay, until the heart is reached. When, however, this organ is invaded, the physical conditions of the environment of the particles are changed. Instead of the quiet viscous flow, hitherto encountered by them, their path is beset by throbbing waves and rapid whirls. Caught in these turbulent vortices, like flotsam in the swirling backwater of a freshet, they are buffeted around, until an ebbing reflux strands them away from the turmoil, in some quiet recess. What becomes of them subsequently I shall consider in a future page. Owing to the distensibility of the pulmonary artery, as shown by Lichtheim, Waller, and others, and to the

thinner muscular walls of the right ventricle as compared with the left, the blood stream at the pulmonary orifice may more closely resemble in its physical conditions the quiet viscous flow of venous blood than the turbulent current at the aortic opening. At all events, many foreign particles appear to evade without difficulty the eddies of this pulmonary Scylla, although they subsequently become entangled in the whirls of the aortic Charybdis.

When incipient atheroma has attacked the aorta, the lesion frequently assumes the semblance of sinuous elevated ridges on the endothelium. These lines or ridges vary in degree of curvature from the annular or spiral form to a slightly undulating streak on the inner surface of the vessel. In some cases the pallid ridges interlace with one another, and form a delicate tracery just above the aortic valve (Plate I. Fig. 9). It is difficult to account for the rings and spirals of atheromatous growths so commonly seen in this situation. If, however, we remember the spiral eddies, which agitate the blood stream at the aortic orifice, may we not consider the close association of liquid vortices with atheromatous tracery in these cases to be more than a fortuitous coincidence? may we not have in the former an important factor in the causation of the spiral ridges?

THE GROWTH OF ATHEROMA.

The fibrous investment—the “inner longitudinal fibrous tissue” of Remak—immediately outside the aortic endothelium, forms, I believe, the basement of a connective tissue network, which binds the various components of the arterial walls together. The basement membrane sends fibrillar strands obliquely outwards at intervals, whence delicate trabeculae are given off—(1) to ramify amongst the meshes—the “fenestrae” of Henle—of the longitudinal elastic layer of the intima, and outside of this to form (2) a loose areolar bed. This bed separates the inner from the middle coat of an artery, and permits free movement between them. Subsequently the network surrounds the components of the middle coat, and ultimately becomes the loose fibrous investment, named the adventitia. During life the meshes of this network are bathed in blood plasma, which is however shut off from the circulation by the endothelium and basement membrane above described. Even during health this plasma may contain a few scattered corpuscles, which are stained by logwood, and basic aniline dyes. If a section is made through an atheromatous patch in the aorta the above structural details can be generally made out, but in addition thereto the endothelium and basement membrane are seen to be invaded by many corpuscles, which stain readily with basic dyes, as did the others. When the disease is in a very early stage these corpuscles are not numerous elsewhere. If the atheroma is more advanced, the corpuscular invasion will have extended outwards through the basement membrane to the tissues beyond,

apparently following, in the first instance, the strands of connective tissue in their oblique passage among the fibro-elastic bundles of the inner and middle coats. Meanwhile the spaces among the fibres, and even the fibrous bundles themselves, will be found to be occupied by members of the invading horde. The former, especially where the tissues are loosely netted, as around the outer surface of the intima, become opened up and enlarged; the latter are split up, more or less completely, into their ultimate fibrils.

The nutrition of the arterial walls is doubtless mainly dependent on the blood plasma, which permeates their interstices. In a healthy vessel the contractility of the media, and the longitudinal plication of the intima at each arterial systole, empty more or less completely this canicular system, and so ensure the frequent renewal of its contents. When dilatation of these cavities occurs, especially if it is accompanied by changes in the elastic lamellæ, and by thickening of the fibrous network, as in early atheroma, the resiliency of the affected tissues must be seriously interfered with, and their nutrition impaired. One of the earliest tissues to feel the secondary effects of malnutrition is the endothelium. The arterial lining membrane at the affected spot undergoes so-called "fatty degeneration," which is never a primary affection in my experience, and in this opinion I am supported by the authority of several eminent pathologists, both at home and abroad; notably by the late Dr. Moxon, and, more recently, by Mr. Timothy Holmes. According to my view fatty degeneration of the endothelium is always preceded by, and associated with a corpuscular invasion, as above described.¹ Occasionally there is hyperplasia of the endothelial cells. In either case the intima is quickly denuded of its inner lining, and the basement membrane is exposed to the blood stream. Another effect of these intermural changes, the result of atheroma, and a purely local one, is a stagnation of the blood plasma within the dilated tissue meshes, especially those in the intima itself, and in the connective tissue between that and the middle coat. This stagnation of the plasma is closely associated with the thickening and stiffening of the inner coat, mainly by a fresh growth of connective tissue. If the wall of an artery is examined at this stage of the disease, the folds and wrinkles of its lining membrane, to which I have elsewhere called attention, are no longer visible, and in their place is a smooth projecting patch of white unyielding tissue. This patch represents the inner layers of the intima, which have lost their pliancy through structural changes, such as those described above. On section we shall find the outer laminae of the intima honeycombed with small holes, in some of which are to be seen one or more corpuscles. At intervals appear cavities containing several corpuscles and fibrillar débris. Sooner or later the walls of

¹ Mr. T. Holmes considers that the "fatty changes in the arteries are preceded by an increased cell-production characteristic of inflammation."—*System of Surgery*, 3rd edition, vol. ii. p. 15.

these cavities, and any projecting fibrils within them, become coated with a granular deposit, consisting of earthy salts;¹ subsequently the granules give way to globular and boss-like projections, until the whole bed of the intima at the diseased part is transformed to a thin bony plate, having on its inner surface, it may be, oblique calcified lamellæ, which pass inward as far as the basement membrane. The latter is usually stretched tightly over the petrified intima, as a fibrous covering; occasionally this fibrous membrane splits and allows a calcareous boss to invade the lumen of the vessel itself. Rarely the basement membrane is converted into a calcified plate, which is extremely thin and brittle. If the corpuscular elements in the affected parts are numerous, the basement membrane, or its representative, rapidly disappears, and the atheromatous ulcer arises. The base of the ulcer is usually the petrified bed of the intima. When an inelastic atheromatous plaque forms a part of the coats of an artery, the vessel frequently becomes dilated at the part. Aneurismal pouches are an occasional although, in my experience, a rare termination of atheroma.

I shall now briefly describe certain modifications of atheromatous growth, only observed upon the cardiac valves. In cases of aortic atheroma the cardiac valves and the endocardium, as is well known, are often affected with the white patches of the disease. The arterial surface of the semilunar valves and the ventricular surface of the mitral are usually attacked in the first instance. If a section of a diseased valve is examined, it will be found to be the seat of a corpuscular invasion. This invasion is associated with a widening of existing fibrous meshes, a new connective tissue growth between the elastic bundles, a splitting up of the latter into their ultimate fibrils, and a consequent loss of pliancy. In other words, subsequent changes in these regions are similar to those observable in atheroma of the arterial walls. Occasionally, however, the disease attacks the line of attachment of a cusp within the Valsalvan sinus. In this case there is a silting up,² so to speak, of the bed of the sinus (see Plate I. Fig. 8), with a contraction of the arterial orifice. On section, wedge-shaped masses of new tissue are found just beneath the floor of the Valsalvan recess. These are composed of corpuscular elements such as those before mentioned, new connective tissue fibres, and many cavities and small holes, within which are visible one or more corpuscles. The new tissues push apart and invade the proper structures of the sinus. In all this we find only an extension of that atheromatous process we have observed in the arterial walls. The subsequent petrification of a similar growth, by a process analogous to that already described, as occasionally taking place in the arterial walls, confirms my

¹ Mr. Rainey, I believe, first drew attention in 1857 to the effect of colloid solutions on crystalline forms, by inducing "molecular coalescence" in the latter. Drs. Guthrie and Ord have also worked at the subject.

² It is possible, as my friend Dr. Adolphus Richardson has suggested, that the systolic murmur heard over the commencement of the aorta in atheromatous disease may occasionally be due to this cause (see Guttman, *Bibliogr.*).

opinion that they are practically identical. The slight differences observable in the two structures, *e.g.* the greater size of the osseous cavities, and the petrification of the whole thickness of the cusp, are mainly due to the anatomical peculiarities of this organ, and not to any essential difference in the manner of osseous deposition. When the ventricular surface of the aortic valve is affected in or near a corpus arantii, either primarily (see p. 6), or as an extension of the disease from the arterial surface, so long as the pliancy of the valve at the line of contact with the aorta is not impaired, we may expect to find a sprouting, warty growth, or vegetation. I shall first consider the case, where the ventricular growth is secondary to atheroma of the arterial surface, and I shall refer to a specimen, which exemplified on the three cusps three successive stages of the process I describe (Plate I. Figs. 1, 4). One aortic cusp of this patient (Case 29 of the Table), just below a corpus arantii was decorated with a single pendulous growth. On section this proved to be a single-celled cavity, having for its walls a continuation of the fibro-elastic layers on that aspect of the cusp (Plate I. Fig. 4). The cavity contained a pale straw-coloured material, which, for reasons I shall give on another page, I imagine to have been of a jelly-like consistence during life. In the cusp there were the usual signs of somewhat advanced atheromatous growth, just before petrification commences. The central parts of the curtain were separated by many interfibrillar cavities in the usual manner, and, I conjecture, the cell within the fibrous pendant was similar in its nature to the cavities. On the second cusp, in a like position, there was a tough warty elevation, at the lower edge of which grew a fibrous flap. The flap in the fresh state could be so adjusted as to cover accurately the projecting part, and the whole then formed a pendant resembling that upon the other cusp. The superficial ventricular layers of the cusp passed into and formed the skin-like flap, which at first must have covered the young sprouts, until their vigorous growth so far stretched their capsule as to produce its atrophy locally. The suction of the left ventricle, to which Dr. Dickinson has recently called attention, effected the final detachment of the flap from its bearings. The third cusp had a somewhat similar rough fibrous vegetation to that just described, but it was without a flap, and its bosses were tipped with soft thready fibrine.

The suction of the auriculo-ventricular cavities during a cardiac diastole tends to produce many special modifications of atheromatous growth on a cardiac valve. The well-established fact that sprouting growths are mostly found on the ventricular surface of the aortic valve, and on the auricular surface of the mitral, points to some physical cause in operation on one side of the valves, which is not obtainable on the other. I must here remind my reader that this remark applies to the growth of an atheroma, and not to its development, which has been discussed previously. In my experience these vigorous sprouting growths, whether they occur on one or the other set of valves, may be

structurally divided into a soft fungating cortical substance, and a central core of fibro-elastic tissue, which passes directly into the proper tissues of the cusp. This latter portion of the growth has the ordinary structure of atheroma, and except for the somewhat larger size of its cavities possibly, and for the greater number of its corpuscles, there would be some difficulty in distinguishing this lesion microscopically from an atheroma of the arterial intima. Out of the central core one or more branches usually pass for some distance into and support the fringe; these branches are also composed of fibrils, which retain in their midst a swarm of well-defined corpuscles, similar to those above mentioned. When, however, the peripheral branches are ultimately reached, the fibrillar structure disappears, and a homogeneous or coarsely granular material takes its place; into this tissue the van of the corpuscular swarm can be traced for a short distance; the corpuscles, then, gradually lose their deeply-stained contour, become somewhat swollen and granular, and eventually disappear, each leaving a slight macula, to mark the spot of its dissolution.

Caught in the swirls of arterial blood, we left the intruding particles for a while (p. 8). I shall now continue their history. I have already drawn attention to the importance of the epithelial lining, as a safeguard against the introduction of foreign particles into the blood; indeed, I imagine the endothelium of the arterial system to be only second to the epithelium, in its power to withstand the assaults of foreign elements, whether they be living or not, provided the attacks are directed against its inner surface. At all events, I have not found microbes within the healthy endothelial cell, although I have repeatedly looked for them there. When, therefore, the intruding particles, much to their discomfort possibly, first find themselves hurried along by the blood stream, they are not in a condition to actively injure the vessels, through which they may pass, even though each one possessed the dozen flagella ascribed by some observers to a typhoid bacillus. Let us, however, assume that a group of noxious bacteria have successfully established themselves in one of the numerous fibrous plications within an aortic Valsalvan sinus. "Cabined, cribbed, confined," within a narrow space, I know not whether these organisms would contrive to thrive and multiply; but I believe that any attempts on their part to force a passage for themselves through the endothelium would be unavailing, so long as that membrane was locally intact. In a majority of cases, doubtless, foreign particles, when conveyed by the blood to some quiet recess away from its eddies, are merely passive agents, and their movements are guided entirely by circumambient currents throughout their intervascular existence. If, then, from any cause, these currents are unavailable, their locomotion at once ceases, yet this event must be a rare one even in a blood vascular recess.

The oft-quoted researches of Metchnikoff upon this subject, confirmed as they subsequently have been by many distinguished

histologists both at home and abroad, need not detain us long in the present instance. The foreign particles, whatever their nature may be, are certainly a superfluous, if not an inimical addition to the blood, and as such ought to be removed thence as soon as may be. For this service nature has detailed certain corpuscles to act as blood scavengers. To these bodies a small group of blood invading particles, which circumstances have either rendered stationary, or endowed with restricted movements, will fall an easy prey. On occasions, as we shall subsequently learn, it may be otherwise.

In the description of the histological peculiarities of atheroma, reference was frequently made to corpuscular swarms, which permeated the affected tissues. I propose now to give fuller details of these minute bodies. Ehrlich pointed out about fifteen years ago that the colourless blood corpuscles, or rather the granules within them, exercised a selective power in relation to aniline dyes. He also showed that in the normal connective tissue the cellular elements found in the network were stained deeply by basic aniline dyes, distinguishing them from many leucocytes (those of the newt excepted). I believe that in a diseased state he admitted the existence of occasional "basophile" leucocytes in the blood of man. However this may be, my own opinion, based on some years' experience, is that in atheroma these "mastzellen" or "nuclear bodies," as I prefer to call them, are blood-born corpuscles, despite their aniline reaction.

Nuclear bodies are small masses of protoplasm, about the size of ordinary leucocytes. Their size is, however, somewhat variable. In fluids (*e.g.* blood plasma) they may assume a spheroidal shape, but within the arterial walls the usual shape affected by them is pip-like or "nuclear." Hence their variable contour, when seen *in situ* under a microscope. In common with their congeners, the ordinary leucocytes, these bodies possess during life the means of individual locomotion by pseudopodial protrusion. Under certain conditions, to be considered hereafter, their activity is apparently increased, and they may appear as minute worm-like bodies. In all cases, when embedded within the tissues, they can be deeply stained by logwood, and basic aniline dyes. Under a high power they are then seen to consist of a lightly-stained plasma, containing deeply-stained granules within.¹ Nuclear bodies are also stained by watery solutions of eosin, but the reaction is due in this case, as in others pointed out by Delépine, to the staining of the plasma instead of the granules. It is, therefore, not a differential stain. Carmine also stains these corpuscles. The most satisfactory stains in

¹ If the distinction, based upon the reaction of certain cell-granules with methylene-blue, is a reliable one, Mr. W. B. Hardy has happened on a differential stain of value in discriminating between "basophile" corpuscles. In *astacus* he found the cells with rose-staining granules lodged in the spaces of a peculiar tissue which forms an adventitia to some of the arteries. In vertebrates they occur to a marked extent in the peculiar adventitia of the blood vessels of the spleen. He regards it as probable that the blue-staining granules are absent from wandering cells.

my hands are a basic aniline solution of Bismarck-brown and Martin-dale's hæmatoxylin solution (Ehrlich).

The removal of a small group of foreign particles from the blood by leucocytes is probably effected with speed and efficiency. Yet the primitive method adopted by these minute organisms of swallowing their obnoxious visitors is undoubtedly open to serious objections from a sanitary standpoint, whenever large numbers are dealt with. Nuclear corpuscles, as met with in atheroma, are, I take it, essentially blood scavengers which have done their work.

The phylogeny of the vascular system in vertebrates has been studied by Bütschli, Ziegler, Hubrecht, and others. Bütschli conjectured that the blood cavity of vertebrates was derived from the segmentation cavity. Ziegler pointed out that the blood and lymph vessels of vertebrates together represent morphologically the primary body cavity in nematodes. The pleuro-peritoneal cavities, on the other hand, have nothing to do morphologically with the primary body cavity. They are formed separately, and are only secondarily connected with the vascular system. The blood vascular system, again, is descended morphologically from the hæmolymphatic system of some invertebrates—arthropods, for instance. It is archicœlomic in its nature. Vertebrate lymph, inasmuch as it is an albuminous fluid containing leucocytes, is more nearly allied to the hæmolymph of many invertebrates than is blood. I conjecture, however, that in the interspaces of an arterial wall, when filled with plasma, we have a close resemblance to the hæmolymph canals which permeate the tissues of arthropods and other invertebrates.

Before continuing the life-history of a nuclear corpuscle, it will be useful to ascertain, as far as may be, by glancing over the animal kingdom, how the blood is cleansed and freed from noxious and effete material in some of the lower classes. Mr. H. E. Durham has described how the amœboid corpuscles of a star-fish, *Asterias rubens*, after devouring some substance which it is to the advantage of the organism to excrete, work their way out through the body wall. In *Asteroidæ* there are no nephridia. In *Clepsine* and *Nephelis*, where they are present, Mr. Shipley states that the nephridial funnels connect the vascular system directly with the body cavity. He confirms Bourne's observations on this point. He has found the nephridial sacs to contain numerous corpuscles from the blood. These amœboid corpuscles "seem to be degenerating, in some cases they appear rather more granular than the normal corpuscles of the blood." It has occurred to him that we have here to do with a similar phenomenon to that observed by Durham; only in this case the blood corpuscles, instead of working their way to the exterior after their meal, "are taken up by the open funnel of the nephridium, and in the sac they disintegrate and are eventually thrown out from the body." In *Hirudinea* and in *Nemertea* the nephridial system, although it is not in all cases in direct

communication with the vascular system, is very closely connected with the blood spaces by its inner ends, which frequently lie within them. Finally, in the vertebrate *Bdellostoma forsteri*, Weldon has found a number of fine tubes anastomosing and running through the substance of the head kidney. These tubules open, on the one hand, into the pericardium, and on the other into a central duct. In this duct was a blood clot similar to those in the blood vessels. We find, then, among the lower animals, at all events, the blood to have very close relations with the nephridia, and that there is good reason for considering one of the functions of these organs to be the removal of disintegrating corpuscles from the blood.

I have already stated my opinion that the nuclear bodies, seen in the atheromatous walls of the artery, are vagrant corpuscles from the blood. It will be expedient to give here, in detail, the grounds for this statement. First, in their relationship to the endarterium, nuclear bodies are most numerous on the inmost layers of an arterial wall, when atheroma is commencing. At this stage of the disease it is by no means unusual to find one or more corpuscles protruding into the lumen of the vessel—corpuscles which, in appearance and in physical properties, are, as far as I can judge, identically the same as those visible in the tissues adjoining. Secondly, these bodies react to stains in a similar manner. Thirdly, in size they closely approximate that of a leucocyte. Other characters, common to the two corpuscular forms, might possibly be mentioned in support of my contention, but space will not allow of their recital. If we admit the nuclear bodies to be the blood scavengers, and to perform their functions by devouring any foreign or effete material within the blood vessels, it follows necessarily that after their objectionable meal they should remove themselves and their ingesta out of the blood as soon as may be. In the lowest forms of life supplied with blood we have seen that the amoeboid corpuscles, whose functions in the economy of the star-fish are closely similar to those of the nuclear bodies in our blood, promptly betake themselves to the outer world through the body walls of asterias, so soon as they have fulfilled their mission as scavengers. By this action they undoubtedly remove, in a simple and efficient manner, any noxious material they may have met with and swallowed. Let us consider whether we can trace any corresponding anxiety in the surfeited corpuscles of our own blood. The manner in which atheroma is developed will furnish an answer to this query. I have elsewhere stated that the arterial endothelium appears to possess in a modified form the capacity of resisting the attacks of micro-organisms, when directed against its inner surface; a resistant power which cutaneous epithelium has acquired in a high degree. Now, although the endothelium may prevent by this means any ordinary attempts at perforation of the arterial walls by a foreign intruder, or even by a vagrant leucocyte in the casual manner ascribed to its Ulyssean wanderings by some writers, it is powerless to check the exodus of a

devoted horde of these scavenging nuclear bodies in an effort to purify the blood at once and effectively from a dangerous intruder.

If a properly-stained section through an atheromatous plash is examined microscopically, the inner endothelial layer will be seen to be invaded by many deeply-stained nuclear bodies. That they are vagrant bodies and not constant inhabitants of the endothelial cells is rendered more probable, among other reasons, by their unequal distribution over the membrane. In many cases two corpuscles are found to have invaded one cell, or several cells appear without a stained nuclear body. In the endothelium these bodies often assume an ovoid or spheroidal shape, as they appear to do in the blood stream. When, however, the tough basement membrane is reached their true wanderings commence, and they mostly assume shapes, expressive of greater activity, such as the pip-like form before alluded to. By the pointed extremity they push aside the elastic and other fibres, and force a passage amid the fenestræ, widening the spaces and loosening the fibres in their outward course. Their exodus through the intima is mainly regulated, as I have suggested elsewhere, by the oblique direction of the strands of connective tissue, which bind together the various layers of elastic and other elements in the arterial walls.

If the above interpretation of the functions of nuclear bodies, and of the manner in which they are carried out, is the correct one, and, of course, I assume that it is so, it will be interesting to inquire, what becomes of these bodies subsequently. This I now propose to do. In describing the histology of the soft tuftlike growths on a cardiac valve I drew attention to the occurrence of gradual changes in the shape and appearance of the nuclear bodies as they approached and passed into the soft tissues of the fringe (p. 13). These changes were ascribed to the gradual dissolution of the corpuscles in the newly-formed fringe. In the specimen to which the description specially applied (No. 51 of Table) the growth began from an atheromatous patch on the ventricular aspect of the mitral valve. From this point the disease had passed, as it usually does in these cases, through the valvular curtain into the pendulous auricular growth beyond. Whilst the ventricular layers of the curtain were overrun by many deeply-stained nuclear bodies, as our eyes passed in review a section of the fibro-elastic strata of the valve, these bodies became visibly less numerous until, upon the auricular side in the newest growth, they disappeared in the manner stated. Now the above account of the progress of the vagrant corpuscle from its home in the blood to its dissolution in some neighbouring tissues, probably represents what actually takes place to many of them after a poisonous meal. And this is an explanation of the presence of maculæ amongst the fibrous layers of an atheromatous plaque, especially in the middle and outer coats of a vessel.

Forcing its way by a leech-like movement through the soft gelatinous plasma, which fills the interfibrillar spaces of the arterial walls, our

blood-scavenger may leave, as it undoubtedly often does, a hollow flattened burrow behind it, wherever the plasma is sufficiently viscous or the fibrillar bands are sufficiently separated to favour this occurrence. These streak-like burrows are common objects in atheromatous sections. At times, haply, the microtometist's chisel may lay open a burrow lengthwise, when the nuclear corpuscle is seen at one extremity (Plate I, Fig. 13, *b*). Having penetrated in this wise to the adventitia of the vessel, and being well away from the blood, which, let us always remember, it is seeking, to use teleological language, to purify by its migration, either its strength fails, or, what is more probable, a reaction is set up between its surface and the surrounding plasma, and the nuclear corpuscle becomes motionless and enveloped in a capsule (Plate II, Fig. 12). And so we have two endings to these migrant bodies, dissolution and encapsulation.

Before leaving this subject I shall briefly state my views in regard to the transformation of the ordinary leucocyte of the vascular system into a basophile nuclear corpuscle of atheromatous tissue. In a recent paper¹ on the development of the lymphatic glands, Dr. Gulland has given a well-selected résumé of what is at present known regarding the nature of leucocytes. He concludes that, although there are many varieties of these bodies, a leucocyte may pass through them all in the course of its existence; that is, I take it, it may possibly start life as a young wandering eosinophile cell, to end its career as a giant stationary cell of the bone marrow, spleen, or embryonic liver. However this may be, it fails to explain why we find so large a proportion of basophile corpuscles in atheromatous tissues or in new connective tissue generally, although they are rarely found within the adjacent blood vessels. I, therefore, offer the following explanation of the phenomena described in the foregoing pages. I have repeatedly insisted on the importance of the function of the nuclear body in atheroma, namely, to withdraw noxious matter from the blood; and I here again allude to it, as furnishing a rational interpretation of the variable reaction of leucocytes to aniline stains. The young leucocyte, the eosinophile cell of Ehrlich and his school, is, according to Gulland, not a phagocyte, at least that is the meaning I attach to his words.² "Microbes are ingested mainly, perhaps, by wandering cells (not eosinophiles), but also by all varieties of the stationary cells." If a nuclear corpuscle, as seen in an atheromatous plash, is a basophile leucocyte, which has made a meal of some noxious blood particles, and has subsequently betaken itself outside the circulatory system, for the purpose and in the manner I have elsewhere mentioned; and if again young leucocytes are for the most part eosinophiles, surely we have good grounds for the assumption that some important change in its constitution may occasionally convert an eosinophile leucocyte into a basophile corpuscle. If the fact of an eosinophile corpuscle having obtained a good round meal—to use homely language—is sufficient to destroy this aniline reaction, and to convert it at once

¹ *Journ. Path. and Bacteriol.*, Edin. and London, 1894, vol. ii. p. 448.

² *Ibid.* p. 458.

possibly into a neutrophile or a basophile corpuscle, I think we have an important clue towards the solution of this physiological problem, namely, that the granular contents of the leucocyte, on which the staining reaction apparently depends, vary with the ingesta. When these ingesta are microbic in their character, and, as a consequence, stain deeply in most cases with basic aniline dyes, the leucocytes become basophiles. With other ingesta, possibly of a less virulent nature, the eosinophiles are changed to neutrophiles. Here, then, we have a fairly simple explanation of the varieties of leucocytes, as shown in their reactions to aniline stains.

THE AFTER-CONSEQUENCES OF ATHEROMA.

Although some nuclear bodies terminate their wanderings within a short distance of the artery they have perforated, we must look upon this operation on their part as an evidence of reversion to an ancestral type of amœboid action rather than as an example of the perfected method adopted by these bodies in the discharge of their functions as blood scavengers to man. I have elsewhere (see Appendix B) alluded to the relationship which appears to exist between atheroma and kidney disease. The table appended to this paper extends and confirms this view. Of the 52 cases therein collected, no fewer than 35 had definite renal lesions, mostly of fibroid type, and in this estimate some cases of simple congestion, and others in which the condition of the renal organs was not stated, have been included amongst the healthy cases. When every allowance has been made for these and other accidents affecting the result, we shall still have the startling fact that, according to this table, more than half of the cases of confirmed atheroma are affected with kidney disease. In atheroma of the aorta, to take the vessel most commonly affected with the disease, the *vasa vasorum* are apparently attacked by the nuclear bodies at an early date. The tissues about the nutrient arterioles are especially beset with numerous vagrant, deeply-stained corpuscles. Now it seems probable that some of these bodies, whilst they are blindly wandering amid the aortic coats, may haply penetrate and re-enter this artery, to be at once swept away unresistingly by the blood stream.

In reviewing the gradual development of the nephridial funnels among the lower animals, there seemed some ground for the conclusion that the chief function of these organs was to collect and to withdraw from the blood those amœboid corpuscles, which, after performing their mission as scavengers, were about to disintegrate.¹ It may prove interesting to inquire briefly, whether our own kidneys assume these functions also. I shall first consider whether the morbid anatomy of a fibrotic kidney, as we find it associated with atheroma, will throw

¹ In the mammals, probably, the process of disintegration takes place within, and is modified by the spleen under ordinary circumstances.

any light on this inquiry. Upon examining a section of the diseased organ, we shall at once notice the uneven distribution of the fibrotic changes amongst the renal tissues. One of the earliest structures involved is the Malpighian tuft, or, more precisely, the membrane covering the tuft, and forming part of Bowman's capsule. This membrane is, at an early stage of the disease, studded with numerous deeply-stained corpuscles, which are physically identical with the nuclear bodies met with in the arterial walls. Subsequently other vascular changes follow; a few only I shall here instance, namely, thickening of the capsules, with shrinkage of the tufts, and greatly increased thickness of the coats of the renal arterioles. These changes take place coincidently with the scattering of numerous groups of deeply-stained nuclear bodies about the necks of the capsules, and in the renal connective tissue. Many of these bodies are encapsuled in strands of newly-formed fibrous tissue, and are mostly spheroidal in shape; others, again, can be found of the distinctive pip-shape, which my readers will remember probably betokens active individual movements on the part of a nuclear body at the time of death. In these changes of renal structure we see possibly the wreck of an organ which has been functionally overtaxed. If we consider the nephridial funnels to have been the precursors of the kidneys in the higher vertebrates, it is probable that the functions of the two organs are similar; and, as a consequence, that the kidneys are concerned in the removal of nuclear bodies, when the latter have fulfilled their mission as blood scavengers. Under ordinary circumstances these bodies will have undergone disintegration before they are submitted to the renal tissues for removal. When, however, owing to the presence of an excessive number of foreign particles in the blood, a correspondingly large number of surfeited corpuscles are also present, the stress of the removal of the surplusage will largely fall upon the kidneys.¹

Referring again to the table, column 3, the observer will find that there are no fewer than 28 cases of pleuritic adhesions noted in this column, exclusive of some cases of hydrothorax. Here, then, we have three-fifths of the recorded cases affected with some, mostly old, pleuritic trouble. Associated with these lesions of the pleural cavities there was in some cases a fibroid degeneration of one or more of the pulmonary lobes. Besides these fibrous changes, incidental to the lungs and pleura, others are noted implicating various organs, yet all consisting essentially in an overgrowth of the fibrous connective mesh-work which binds the different parts of an organ together. MM. Duplaix and Isnard have at different times drawn attention to this "sclerotic process" in connection with vascular changes, that is, with

¹ Drs. Jacob and Krüger, in their recent experiments on a case of leukæmia, showed that an increase in the nitrogen of the uric acid and nuclein bases of the urine is associated with the increase in the number of leucocytes.

a luxuriant growth of areolar tissue around the vessels, especially around the central arteries. From what has been stated of the rôle assumed by the nuclear corpuscle, in the production of the sclerotic changes of atheroma, it seems probable that these bodies in some mysterious way are directly concerned in the production of all such overgrowths of connective tissue.¹ For whether we observe the young tissue strands on the thickened Glisson's capsule, in the interstices of a granular kidney, or amongst the meshes of an atheromatous aorta, we shall find the same basic staining corpuscles everywhere interspersed amid them.

In considering the passage of foreign particles through the blood vessels, I suggested that the transit was mostly performed by coherent groups of these elements, and reasons were given in support of this view (p. 8). If the facts of the case are here correctly stated, it follows that when foreign matter finds an entrance to the vascular system by any portal within the alimentary tract, the particulate group must pass through the capillary network of the lungs before it arrives at the aorta. In many cases this passage, owing to the small size of the group, or to the mobility of its constituent parts, is readily effected. It must, however, occasionally happen that the group becomes impacted in the lungs. Then nuclear bodies at once strive to remove the obstruction. The engorged corpuscles on the cardiac side of the obstruction may or may not subsequently pass along the pulmonary veins to the left heart, those on the far side will have every inducement to penetrate the vessel's walls, a privilege of which they readily avail themselves. And so they eventually find their way into and increase the fibrous stroma of the lungs, producing puckering of the alveolar tissue, or even fibrous adhesions between the pleural surfaces, if their path leads in that direction. It is often a matter of surprise to find, after death, extensive valvular lesions, or atheromatous ulceration of the aorta, without any secondary thrombi, such as we might be led to expect, in the peripheral vessels. I believe in these cases, many interesting examples² of which have been from time to time published, the sequel of pathological events may be explained as follows:—The detritus of the vegetative growth or atheromatous plaque, as the case may be, consists in these instances of finely-divided particles. These "orts," when they obstruct a vessel, are entirely devoured within a short period by nuclear bodies. Living nuclear bodies, I contend, never produce thrombi *per se*, their province is to remove them when discovered. When thrombi occur during life there is strong presumptive evidence that the blood vessels have been invaded by foreign particles, which by obstructing the flow of blood in a vessel have induced clotting and the formation of a thrombus. In nuclear bodies, then, we shall find not only the true blood scavengers, and the producers of atheroma, but

¹ Ziegler and Marchand drew attention to this subject in 1891.

² See papers by Drs. Church, Moxon, and others (Appendix B).

also the probable originators of fibrous overgrowth, in whatever part of the body it may appear.

Finally, my thanks are due to Drs. W. S. Church and Adolphus J. Richardson for their kindness in perusing the MS. and for several valuable suggestions regarding it. I am also much indebted to *Virchow's Jahresberichte* for the able abstracts of many of the medical papers referred to in this essay.

DESCRIPTION OF PLATES I. AND II.

- FIG. 1.—Two of the aortic cusps from Case 29. At the lower part of the right corpus arantii is a tuberculated growth with a fibrous flap attached below. In the fresh state the flap could be adjusted to cover the growth. The case is fully described (p. 12).
- FIG. 2.—Fringe-like growth springing from the base of a corpus arantii, and extending along the border of the lunule.
- FIG. 3.—Fibrous folds on the ventricular aspect of aortic cusp affected with atheroma (p. 6).
- FIG. 4.—Section of the third aortic cusp, with a pendulous growth attached, from Case 29. Fig 1, (a) the growth. $\times 25$.
- FIGS 5 and 6.—Sections of the atheromatous tissues highly magnified, showing the dissociation of the fibrous elements, and the widening out of the meshes which follow an invasion of nuclear bodies.
- FIG. 7.—Section through an aortic cusp, thickened at (a) by atheromatous deposit; at (b), on the arterial side, is a recess, the seat of commencing atheroma.
- FIG. 8.—Section through a diseased Valsalvan sinus. The bed of the sinus is, as it were, silted up by the interposition of wedge-shaped pieces of tissue (b), between the cusp (a) and the aorta (c). Case 45. \times about 3.
- FIG. 9.—The arterial surface of an aortic cusp attacked with atheroma.
- FIG. 10.—A part of the aorta just above a cusp stained slightly by eosin, showing circinate patches of atheroma. Case 46. $\times 2$.
- FIG. 11.—Section of the aortic intima showing the passage of nuclear corpuscles obliquely outwards. Ehrlich's hæmatoxylin solution. \times about 500.
- FIG. 12.—Section of a renal artery, highly magnified, showing a nest of nuclear bodies encapsuled between the layers of the media and the adventitia. $\times 1350$.
- FIG. 13.—Nuclear bodies highly magnified, *in situ*. a. Nuclear body surrounded by jelly-like plasma, in which fine fibrils of new connective tissue are visible. b. Nuclear body at the extremity of a burrow. c. Nuclear body in the act of worming its way through the plasma at death. $\times 1350$.
- FIG. 14.—Section through the intima of the aorta, showing the oblique fibres trending outwards. aa. Endothelium. The fibres pass from the basement membrane (b') towards the right (b). From a photograph.
- FIG. 15.—Atheroma of aorta with contracted fibrous kidneys.



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5

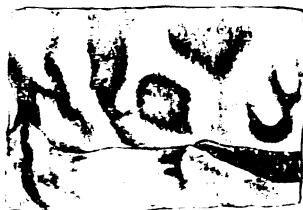


Fig. 6



Fig. 7



Fig. 8



Fig. 10



Fig. 11

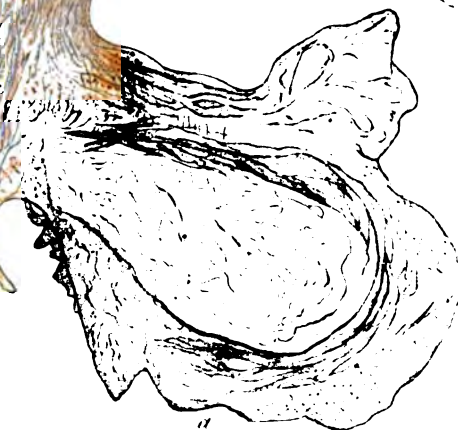
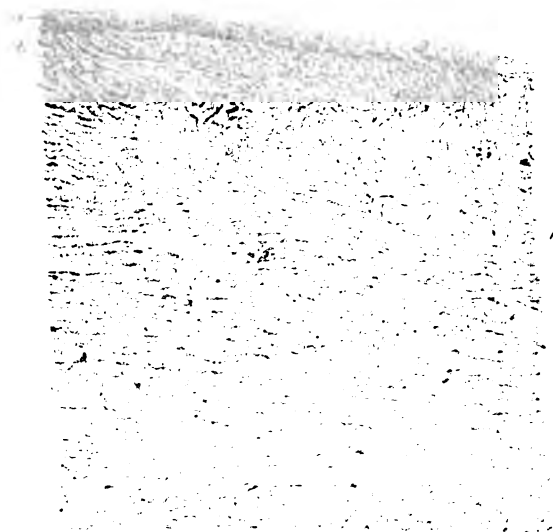
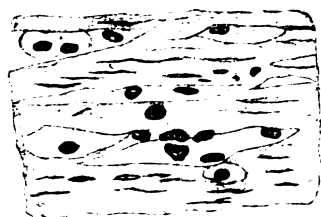


Fig. 12

TO VISA
AIRPORT



to you
Angela

APPENDIX A.

Table of Cases of Atheroma showing various Diseases with which the chief Organs were concurrently affected.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys.	Other Organs, History, Remarks, etc.
No. 1, F., 55, cook.	Aorta somewhat atheromatous.	Left, œdematous; no vomicæ; pleural surface studded with cancerous growths.	Left, mottled; capsule adherent.	Peritoneum, omentum, and mesentery thickened, and infiltrated with like deposits.
No. 2, M., 47, bricklayer.	Atheroma of mitral valve and ascending aorta; aortic cusps perforated by 2 or 3 small punctures along free border.	Right pleura universally adherent; lungs congested and œdematous.	Large, much congested; urine, non-albuminous.	A glioma in left cerebral hemisphere.
No. 3, F., 50.	Vegetations on 2 aortic cusps; thrombi in both pulmonary arteries, adherent to walls and nearly occluding vessels.	Middle lobe of right lung greenish brown in patches; both lungs œdematous.	Capsules adherent; granular; cortex thin, streaky.	Gall-stones, malignant disease of liver and gall-ducts.
No. 4, F., 38.	Thoracic aorta converted into rigid calcareous tube; heart and abdominal aorta healthy.	Patient died of malignant disease of the liver. (S. C. H. Museum, C. 58.)
No. 5.	One or two cystic bodies on thickened edge of mitral; 2 aortic cusps coherent; abdominal aorta, a calcareous tube; ascending aorta atheromatous and dilated.	Firm pleural adhesions, over right lung; at left apex small vomicæ; a few vascular tumours size of a swan-shot in lower lobe.	Left, converted to fibrous mass, weight, $\frac{3}{4}$ oz.; right, 7 oz., healthy; both ureters normal size.	Numerous vascular growths in liver, gall-bladder, and beneath peritoneum; a large ragged ulcer occupied cardiac end of stomach.
No. 6, M., 44, plasterer.	Aneurism of ascending aorta involving pericardium; muscular tissue of heart soft, valves healthy.	Right pleura held 50 oz. clear fluid; left pleura obliterated by firm adhesions; right lung collapsed.	Capsules firmly adherent; finely granular surface.	Liver firm, congested; weight, 3 lb. 1 oz.
No. 7, F.	Aneurism of arch; much atheroma beyond.	Left lung collapsed; left pleura contained some pints of pus.	Right, surface very granular; both fibrous; weight, 14 oz.	Uterus contained a fibroid.

APPENDIX A.—*Cases of Atheroma*—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys.	Other Organs, History, Remarks, etc.
No. 8, M.	Heart, 37 oz.; left ventricle admitted fist; posterior aortic cusp perforated by an ulcer; arteritis.	Pleuræ adherent in parts.	Healthy.	Patient had had syphilis severely.
No. 9, M., 20, costermonger.	Fusiform aneurism of aortic sinus; inner coat of aorta very atheromatous in parts.	Both pleuræ contained moderate amount of clear fluid; some old adhesions, right apex.	Not examined.	Cicatrix of an old bubo in right groin.
No. 10, M., 56.	Atheroma of aorta and of arteries of lower limbs.	...	Shrunken.	History of syphilis. (See R. Johnston, <i>Path. Trans.</i> 1890, vol. xi.)
No. 11, M., 56, painter.	Heart, 10½ oz.; aortic valves converted into two tubercular flaps; much atheroma around.	Some threadlike adhesions at apices; left congested, oedematous.	Left, cystic and fibrous; a few miliary caseous bodies.	A large sloughing abscess of scrotum, extending beneath rectus abdominis; several false urethral passages.
No. 12, M., 55.	Aneurism of ascending aorta, bursting into pericardium; aorta very atheromatous above.	Left, congested; oedematous.	Hard and congested; weight, 10½ oz.	Brought in dead.
No. 13, M., general dealer.	Ecchymoses beneath endocardium; pulmonary arteries very atheromatous.	Bronchi, especially of left lung, dilated; upper lobes fibrous; visceral pleuræ semi-cartilaginous, thick.	Weight, 11 oz.; congested, ecchymoses on surface.	Left testis converted into fibrous mass; left epididymis seat of caseous substance.
No. 14, M., 55, clerk.	Several small hæmorrhages in pons; one small cyst in funiculus cuneata; cerebral arteries atheromatous.	Numerous patches of thick pleuræ at apices; left lung compressed by fluid in pleura, in part carnified; no tubercle.	Granular, cystic.	Many miliary fibrous deposits on surface of spleen, none within; history of rheumatic gout.
No. 15, M., about 65.	Large hæmorrhage in left ventricle; aorta, cerebral arteries, etc., atheromatous; heart, 1 lb.; hypertrophied.	...	Right, ½ oz.; left, 6 oz. Right, congenitally undeveloped; left, cystic, fibrous. Urine, 1 per cent. albumin.	Seized with right hemiplegia 14 days before death.

APPENDIX A.—Cases of Atheroma—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys.	Other Organs, History, Remarks, etc.
No. 16, M., 44, musician.	Two aortic cusps calcified, third fringed with soft growth; cerebral arteries atheromatous.	Pleuræ adherent generally; lungs airless and congested at bases.	Congested, tough, capsules adherent.	(See S. B. H. Reports, vol. xxviii. for history.)
No. 17, M., about 40, collector.	Extensive laceration of brain by hæmorrhage; cerebral arteries very atheromatous; aorta also atheromatous.	...	Small and granular.	Died shortly after admission.
No. 18, M., 25.	Thoracic aorta atheromatous; pulmonary arteries dilated; pericardium adherent.	Right lung coated with thick layers of flaky lymph; left pleura held much fluid.	Large, and very tough and congested.	A large amount of tubercle dotted over peritoneum; tubercular testes.
No. 19, M., 36.	Some atheroma at the commencement of the aorta.	Several vomicae in left lung; surrounding tissues solid, small white granules scattered over rest of lung; large vomicae in right lung.	Large and pale, capsules peeled well; pyramids red, well defined; cortex about normal. Urine very albuminous during life.	...
No. 20, M., 54, groom.	Three or four ulcers in ascending aorta; transverse arch very atheromatous.	Pleuræ adherent at apices; large vomica, right lung; fibroid thickening around.	Mottled, shrunken, cystic.	Mucous hæmorrhages from nose and mouth; anasarca; liver fatty.
No. 21, M., 37.	Heart, 22 oz.; dilated and left chambers hypertrophied; aorta atheromatous.	Right lung, two or three cavities in lower lobe; left lung consolidated at base, fibrous tissue thickened; numerous softish adhesions.	12 oz., granular; urine very albuminous during life.	Liver capsule thickened; discrete, glistening; white granules in Sylvian fissure and over pia mater.

APPENDIX A.—*Cases of Atheroma*—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleura.	Kidneys.	Other Organs, History, Remarks, etc.
No. 22, M., 28, clerk.	Thoracic aorta atheromatous throughout; heart healthy.	Many small vomices with puckering at left apex; pleurae much thickened in parts; no tubercle bacilli found in sputa.	Weight, 1 lb. 2 oz.; amyloid; capsules adherent; finely granular surface.	Lower two-thirds of small intestine, and the large intestine dotted over with ulcers, whence pus could be expressed; amyloid reaction to m. m.
No. 23, M., 24, watch finisher.	Aorta atheromatous; heart healthy.	Firm pleural adhesions; both lungs contained vomices with increased fibrous tissue in parts.	Weight, 19 oz.; cortex pale; pyramids red.	Many suppurating ulcers over Peyer's patches.
No. 24, M., 53, policeman.	Aorta atheromatous; heart healthy.	Several vomices in both apices; masses of tubercle elsewhere, with adjacent fibrous changes; thready pleural adhesions.	Weight, 6½ oz.; granular.	Many intestinal ulcers; several pints of ascitic fluid in abdominal cavity.
No. 25, M., 55, joiner.	Heart, 14 oz.; pericardium adherent to surrounding organs; serous surface covered with lymph; aorta atheromatous.	Pleural cavities obliterated; scattered miliary tubercles throughout both lungs; cavity, size of a hen's egg at left apex.	Weight, 11 oz.; yellow miliary bodies in substance; capsules stripped readily.	Many intestinal ulcers; bronchial glands enlarged.
No. 26, M., 21, barber.	Some hypertrophy of left ventricle; aorta atheromatous.	...	Capsules somewhat adherent; tough.	Appendix vermiformis had sloughed; purulent fluid in abdominal cavity.
No. 27, M., 11, scholar.	Aorta for an inch above valve atheromatous.	...	Congestive mottling.	The loose end of a sloughed appendix was the seat of an abscess.
No. 28, F.	Some patches of atheroma in ascending aorta; heart healthy; left brachial, subclavian, common iliac veins occluded by firmly adherent clots.	Fluid in both pleurae; also firm adhesions at apices.	Weight, 8 oz.; granular.	Abscess cavity in the integument above the pubes; anasarca.

APPENDIX A.—Cases of Atheroma—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys.	Other Organs, History, Remarks, etc.
No. 29, F., 36.	Fringes of soft blood-stained, vegetation on mitral; aortic valve also affected; aorta atheromatous.	...	Capsules thickened; granular, congested.	An abscess cavity at the back of right ovary, shut off from peritoneum.
No. 30, F., 36, barmaid.	Adherent pericardium; aorta atheromatous; right jugular and innominate veins occluded by clots during life.	The upper lobes of both lungs dull red, solid, and firm; recent pleurisy at each apex.	Capsules adherent; surface granular; urine albuminous.	Liver capsules thickened; history of rheumatism, 12 years ago; anasarca general for two weeks before death.
No. 31, F., 43.	Warty growths on mitral valves, opening annular; thrombi in uterine veins.	Right pleura distended with fluid; a patch of intense congestion at base.	Weight $\frac{1}{2}$ lb.; many cysts; urine albuminous.	Fibroid of uterus; anasarca; history of rheumatism.
No. 32, M., 14, machine-boy.	Fibrous growths on pulmonary and aortic valves; chambers dilated and hypertrophied.	Left pleura universally adherent; lungs oedematous and congested.	Irregular congestion.	No history of acute rheumatism; suffered from rheumatic pains; family history cardiac.
No. 33, M., 35.	Aortic mitral curtain seat of ulcer, $\frac{1}{2}$ in. diameter, covered with soft vegetation on ventricular aspect.	A few friable adhesions in both pleuræ; left, lower lobe solid, Temp. 104° before death.	Congested.	Acute rheumatism, four attacks; left hemiplegia 14 years ago for 10 weeks; no emboli observed.
No. 34, F., 52.	Two small ulcers on ventricular aspect of mitral, which was "buttonhole" in shape and calcified.	...	Capsules adherent; surface pitted; cortex thin.	History of acute rheumatism 30 years ago; thighs brawny; fingers with ulnar trend; anasarca, twelve months.
No. 35, F., 30, servant.	Heart, 6 oz.; third aortic cusp congenitally undeveloped; some atheroma of aorta; pericardial effusion considerable.	Both pleuræ contained much serum; lungs carnified in parts.	Weight, 7 oz.; tough; pyramids dark blue; cortex thin. Urine seldom albuminous.	Family history of heart and dropsy; facial palsy 12 months ago.

APPENDIX A.—Cases of *Atheroma*—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys.	Other Organs, History, Remarks, etc.
No. 36, M., 23, carter.	Heart, 30 oz.; pericardium adherent; mitral and aortic valves thickened.	Many fibrous adhesions to pleuræ.	Not examined.	History of three attacks of rheumatism.
No. 37, M., 34, gardener.	Small rough vegetations on free edge of mitral; granulations dotted over pia mater.	...	Not examined.	History of rheumatic pains in joints; basal ganglia soft.
No. 38, M., 16, errand-boy.	Heart, 17 oz.; adherent and thickened pericardium; tricuspid, mitral, and aortic valves roughened; two last with ulcers.	Pleuræ contained about 3 pints of serum; lungs collapsed at bases, cedematous.	Weight, 7 oz.; shrunk, mottled; urine very albuminous, 70 oz. daily.	Two years ago had stone removed from urethra; pericarditis subsequently; pains in joints without swelling; temperature high.
No. 39.	Heart, 1 lb. 2 oz.; some atheroma of mitral valve and of aorta.	Lungs emphysematous; mucous membrane of colon pigmented and mammilated.	Many ecchymoses; some shrinkage.	Liver, 4 lb. 2 oz.; several cysts in substance filled with bile.
No. 40, F., 33.	Heart, 18 oz.; mitral valve thickened, contracted, and aortic valve fringed with vegetations; no ulcers.	Lungs shrunk and collapsed at bases; old adhesions.	A little cloudy swelling of cortex; two infarcts in right kidney.	Gall-stones impacted in cystic and common ducts; liver ducts dilated and filled with bile; acute rheumatism 12 years ago.
No. 41, M.	Heart, 28 oz.; extensive calcareous changes at both mitral and aortic orifices.	Some effusion into both pleuræ; lungs congested.	Large, hard, dark, 16 oz.; urine, non-albuminous.	Liver nutmeggy; no history of rheumatism nor syphilis; anasarca.
No. 42, M., 32, retired soldier.	Aortic valve converted into a calcareous ring with a central slit-like opening.	Both pleuræ contained fluid; some emphysema.	Intense congestion; cortex thin.	Anasarca 2 years; no history of rheumatism.
No. 43, M., 67, tutor.	Aorta and pulmonary artery very atheromatous; mitral thickened.	Firm adhesions over both lungs, which were emphysematous and cedematous.	Small, congested, granular.	Ill 2 years with bronchitis and asthma.

APPENDIX A.—*Cases of Atheroma*—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleura.	Kidneys.	Other Organs, History, Remarks, etc.
. 44, M., 62, labourer.	Heart, 20 oz.; mitral and aortic valves thickened and puckered; atheroma of aorta.	Each pleura contained about 1 pint of serum; some old adhesions, some cedema of lungs; no bronchial dilatation.	Weight, 1 lb.; capsules thickened; cystic granular; surface pale.	Anasarca, diarrhoea, anaemia, 15 months; no hæmorrhages.
. 45, M., 5½.	Aortic valve incompetent through shortening of cusps, and silting up of sinuses (see Plate I. Fig. 8).	Congested.	Tough and congested.	Illness began with fit, 9 months ago; his limbs were swollen and painful at intervals.
. 46, F., 18, servant.	Aorta just above the valve covered with patches of atheroma.	Severely burnt 10 days before death.
. 47, M., 41, servant.	Heart, 20 oz.; dilatation and hypertrophy of left ventricle, valves healthy; pericardial fluid, 5 oz.; renal arteries patulous.	Pleura contained 25 oz. of clear fluid; apices of lungs adherent and puckered; bases collapsed.	Weight, 13 oz.; capsules adherent; sub-stancedark, flexible; papillæ shortened; urine very albuminous, scanty.	Had delirium tremens and droopy two years ago.
. 48, F., 63.	Advanced atheroma of basal cerebral arteries.	(See Joffroy, App. B.)	...	Peripheral neuritis with right hemiplegia, and softening of left inner capsule.
. 49, F., 17, ironer.	Heart cavities dilated, muscle pale; streaks of atheroma above aortic valve.	Right lung covered with flaky lymph and solid, in colour greyish yellow; left lung cedematous.	Large, bases of pyramids very congested, elsewhere pale; urine albuminous.	Ill with typhoid symptoms, diarrhoea and lung mischief, with high temperature two weeks before death; no intestinal ulcers.
. 50, F., 24, ironer.	Heart dilated; numerous patches of atheroma at the commencement of aorta.	Left pleura filled by a globular mass of tissue firmly adherent to walls; lymphadenoma; left lung pushed downwards.	Large, congested, tough.	Pleuritic effusion on left side 12 years ago; in delicate health subsequently.

APPENDIX A.—*Cases of Atheroma*—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleura.	Kidneys.	Other Organs, History, Remarks, etc.
No. 51, F., 58, matron.	Mitral valve almost occluded by two soft conical growths; the myocardium was soft and friable; aorta atheromatous.	Both lungs dotted over with petechiæ; no adhesions; very oedematous and congested at bases; no infarcts found.	Cortex very thin; capsule adherent and thick; granular, cysts; urine held blood.	Admitted in a semi-conscious state, pulse high tension, limbs oedematous; illness began 5 weeks before admission with diarrhœa; spleen contained many small abscesses; a small abscess was also found just to the outside of the bend of the left internal capsule (size of a nut).
No. 52, F., 2½.	Some streaks of atheroma just above aortic valve.	Patches of bronchopneumonia in both lungs.	Very congested; urine very albuminous.	Diphtheritic laryngitis; tracheotomy.

APPENDIX B.

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REMARKS ON THE HEART IN DEBILITY.

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SEVERAL years ago considerable interest was shown in certain discussions as to the cause of the clinical facts observed in cases of cardiac debility. The principal subjects under debate at that time were the explanations which had been advanced regarding two of the phenomena commonly observed in feeble conditions of the heart. The first of these, and that the more frequent in its occurrence, is the systolic murmur heard in the second left intercostal space, at or near the pulmonary area; the second, not so often presenting itself to the observer, is the systolic impulse seen and felt in the same locality. To the investigation and explanation of these appearances several observers devoted much attention, and, inasmuch as many of the points connected with the physical signs under discussion were virtually settled, it may seem unnecessary to bring the subject forward again. As one of those, however, who took part in the discussions on this question, it seems to me nothing more than simple justice to those whose views then differed from my own to state frankly and candidly the opinions which have been borne in upon me since the time referred to. In attempting to do so, it will be my endeavour to be as brief as possible, and, in particular, to avoid unnecessary reference to older observers. This may be done the more easily as William Russell has given a very complete and most masterly summary of the views of previous authors in his work on this subject.

For the present purpose it is only necessary to recall a few facts. In order to account for the systolic murmur and accompanying pulsation sometimes seen in the second left intercostal space, in cases of mitral incompetence, Naunyn advanced the hypothesis that both appearances are produced by the backward stream from the left ventricle into the left auricle. According to this view the systolic murmur is of mitral origin, and is conducted by the regurgitant current into the dilated left auricular appendix, while the pulsation is caused by the same stream distending the appendix and thereby producing an impulse

on the thoracic parietes. Balfour applied this hypothesis to the corresponding phenomena, so commonly seen in the feeble heart of anæmia and allied conditions. In his work on diseases of the heart, as well as in separate papers dealing with this special question, he has strongly advocated this explanation, and his opinions were warmly supported in some contributions made at the same time by myself. We were, however, unable to adduce any evidence obtained from morbid anatomy in favour of our views, and, although many of the clinical features appeared to be explained by them, they could not be regarded as resting on any sure pathological basis.

Russell brought to the elucidation of the questions under discussion a large number of clinical and pathological observations, from the consideration of which he came to very different conclusions. He showed that in many cases where the systolic pulsation in the second left intercostal space had existed before death, post-mortem examination proved that the impulse could only have been caused by the conus arteriosus, which, in consequence of dilatation of the right ventricle, was so far to the left as to occupy the site of pulsation in the left intercostal space between the second and third cartilages. With regard to the basic murmur, heard in cardiac debility, Russell proposed two explanations. He suggested that in some cases it might be produced by dilatation of the left auricle, which, pressing upwards upon the pulmonary artery, gives rise to a narrowing of its lumen, while in other cases it is simply the systolic murmur of tricuspid incompetence propagated upwards to the conus arteriosus.

The main points at issue in the discussions regarding this subject were very critically examined and judiciously weighed by Bramwell in his systematic work. As the result of a very careful review of the arguments which have been advanced by Balfour and Russell, he rejects the theories of both with regard to the production of the basic systolic murmur, and attributes it to the sudden pulsation of a large blood wave of abnormal composition into the vessel, which he thinks may probably be dilated.

Handford holds that the pulmonary systolic murmur, which he describes as disappearing in the erect position and reappearing when the patient is recumbent, is produced by the pressure of an enlarged, flabby, and dilated heart on the pulmonary artery.

Foxwell has, like Russell, found the pulmonary artery to be displaced considerably upwards. He regards the murmur in the pulmonary area as caused by a complicated change in the shape and position of the pulmonary artery, whereby its curve becomes increased, its axis and that of the right ventricle lie at a different angle from that existing under healthy conditions, and the vessel is flattened against the aorta. At the same time, however, he accepts Russell's view of a distended right auricle as the cause of the murmur in some cases.

In the last place, Sansom, after an examination of the views of

Balfour and Russell, which leads him to dissent from both, advances the opinion that the basic murmur can be initiated at the over-strained portion of the right ventricle, the conus just below the pulmonary valves, by the production of a fibrillar tremor. He is, however, also inclined to believe that the cusps may themselves vibrate in the current.

It is easy for me now to consider the questions involved in a perfectly dispassionate and impartial spirit, inasmuch as Russell has, in my opinion, absolutely disproved the views of all observers previous to himself. He has demonstrated, beyond all possibility of doubt, that the left auricle never reaches the anterior wall of the thorax, and that the pulsation in the second left intercostal space is produced by the conus arteriosus. The observations of Foxwell, Harris, and Mackenzie support him in this, and it seems to me that the explanation of Naunyn and Balfour has been entirely refuted.

This decision leads of necessity to the further conclusion that the hypothesis of Naunyn and Balfour with regard to the origin of the systolic murmur heard in the pulmonary area falls to the ground, for since the left auricle never approaches the surface there is no medium for the conduction of a mitral murmur towards the base of the heart. But it must further be stated that, in a large proportion of cases, there is no evidence of any mitral incompetence, and that it is a mere begging of the question to assume it.

The view advanced by Bramwell may be regarded as in every respect a compromise, as Russell puts it, between the explanations of Hope and Beau, and it has, like their theories, been effectually disposed of by him.

But while granting freely that Russell has disproved all previous theories, it seems to me that part of his own explanation will not bear investigation. He has yet to prove that in early stages the left auricle is dilated. In truth, the conditions appear to be the very reverse of those which he postulates. The mitral cusps are very often perfectly competent, and as long as there is no mitral regurgitation the pressure in the pulmonary artery must be greater than that in the left auricle.

The explanation of Handford cannot be accepted, not only because the basic murmur is heard very frequently indeed while the patient is in the erect position, but also because it makes its appearance before there is any noteworthy enlargement of the ventricles. The same argument applies to the reasoning of Foxwell with equal cogency, while his experiment of forcing water into the right ventricle of a debilitated subject after tying the pulmonary artery is so unlike anything in nature that it cannot be held to prove anything.

Sansom, finally, is obviously in error, as, if over-strain of the ventricle were a valid cause for a murmur, such a phenomenon would be of much more common occurrence than is the case. Almost every case of chronic renal cirrhosis, for example, would be attended by a murmur produced by the strain thrown on the left ventricle.

In order to present the clinical features with which this paper is

concerned, in a concrete form, the following case is worthy of record. It has already been utilised for the illustration of another point in a paper recently published, which is in no way directly connected with the object of this contribution.

MAGGIE G., æt. 18, unmarried, engaged in household duties; was admitted to Ward 25 of the Royal Infirmary on 5th June 1893, complaining of pains in her wrists and elbows.

Her father and mother, both æt. 42, had always been in good health. She had four brothers and one sister, all very strong, but three brothers had died in infancy. The patient's social surroundings had always been good.

She had never been very robust, and four years before admission had suffered from a rheumatic attack, since when she had never felt very well. About four months before entering the Hospital, pains had begun in the joints and had persisted ever since.

On her admission the patient was found to be somewhat pale, with a bright spot on each cheek. The skin was moist. The temperature was normal. The tongue was slightly furred, but the digestive system was otherwise healthy. There was no symptom connected with the hæmopoietic system.

She complained of some palpitation and a slight degree of breathlessness. The pulse was of low tension and moderate volume, perfectly regular, and varying in rate from 80 to 90. There was some pulsation in the veins of the neck, and a very distinct impulse in the second left intercostal space. On palpation the apex beat was found to be in the fifth left intercostal space, $3\frac{1}{4}$ in. from midsternum. The pulsation, systolic in time, in the second left intercostal space was found to be most distinct, $1\frac{1}{2}$ in. from the midsternal line. A tracing obtained from it by means of a revolving cylinder is given in the



FIG. 1.

accompanying figure (Fig. 1). No thrill could be detected over any part of the præcordia. The cardiac dulness extended to 1 in. to the right and 4 in. to the left of the middle line at the level of the fourth rib. On auscultation, a venous hum was heard in the neck, and there were murmurs, systolic in rhythm, over the whole præcordia, which, on careful analysis, proved to be twofold. Around the region of the apex beat, and with its maximum loudness in the fourth interspace $3\frac{1}{2}$ in. from midsternum, there was a harsh blowing systolic murmur, conducted as far as the edge of the sternum to the right, and beyond the anterior axillary line to the left. Over almost the entire sternal region there was a soft blowing systolic murmur, quite different in character from that heard at the apex. It had the same tone throughout the whole sternal region, but it seemed to have two points of maximum intensity—to be

more exact, it was loudest in the pulmonary region, exactly over the area of pulsation, from which point it waned in its intensity in every direction until near the lower end of the sternum, when it became louder, again culminating at the point where the left side of the sternum was joined by the sixth costal cartilage; but in this situation the murmur was not quite so loud as over the area of pulsation in the pulmonary region. The second sound was frequently reduplicated, and the later of the two second sounds, which could by auscultation be determined to be that due to the pulmonic cusps, was instantly followed by a short, sharp, high-pitched murmur, perfectly soft in character. This murmur was extremely restricted in its distribution, being only heard over a small triangular area $2\frac{1}{2}$ in. in vertical and 2 in. in horizontal measurement, extending along the left border of the sternum, from the lower border of the third costal cartilage to the upper border of the fifth. This murmur was perfectly soft in character, and was absolutely unlike the obstructive diastolic murmur which is found in mitral stenosis. It could not be due to aortic

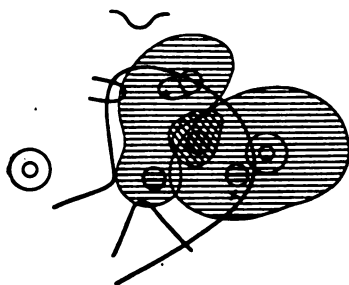


FIG. 2.

disease, of which there was no indication, and it could only, therefore, be a murmur of regurgitation, from the pulmonary artery into the right ventricle, due to the increased pressure and consequent dilatation of the orifice, with relative and transient incompetence of the cusps. The production of this murmur has been discussed by me in the paper referred to. All these murmurs are shown in Fig. 2, in which the areas over which the systolic murmurs were audible are marked by horizontal lines, while the diastolic murmur is shaded diagonally. The maximum intensities of the murmur are

shown by the circles darkly shaded, and the impulses by the crosses.

The other systems presented no symptoms of disease, with the sole exception of a few crepitations at the bases of both lungs.

The diagnosis was cardiac dilatation, with mitral and tricuspid regurgitation, produced by the febrile affection, but it was considered probable that some stenosis of the mitral orifice might be insidiously progressing, although this was a mere supposition, not based on any direct evidence. The crepitations at the bases of the lungs were regarded as the expression of passive congestion from mitral incompetence, and the diastolic murmur was assumed to be one of pulmonary escape, in consequence of the strain on the artery from the high pressure within it.

By means of salol and similar remedies the patient was relieved of her rheumatic symptoms, and the administration of iron with other tonics greatly improved the cardiac condition. The diastolic murmur disappeared, and the lungs cleared up, but at the time of the patient's departure from the Royal Infirmary, on 17th July 1893, she still had the systolic murmurs, and the pulsation in the second intercostal space. She presented herself at the Hospital on the 2nd March 1894, when the diastolic murmur was found to be still absent, but the systolic murmurs were present as before. The first sound in the mitral area, preceding the systolic murmur, was, however, loud and clanging in

character, which seemed to support the view that a stenosis of the mitral orifice was gradually developing.

This case brings into prominence the systolic impulse in the second left intercostal space, as well as the systolic murmur in the same position. From the physical signs there could be no doubt of the presence of mitral and tricuspid incompetence, and it may be remarked here that the pulsation in the second left intercostal space is never observed except in cases which present so much dilatation as to allow of regurgitation at both auriculo-ventricular orifices. The diastolic murmur, which has been very fully discussed in the paper referred to, seems to me to be caused by regurgitation at the pulmonary orifice from high pressure and relative incompetence, and requires no further remark in this place. The systolic murmur heard over the sternal region of the præcordia appears to be the same throughout with two points of maximum intensity, and seems to me easily explained in this way, that, while at the lower end of the sternum it is heard with great distinctness, owing to the proximity of the muscular wall of the right ventricle and of the tricuspid valve, it is also heard with at least as much intensity over the conus arteriosus. It seems to me, in short, to be purely a murmur of regurgitation at the tricuspid orifice. While Russell's view with regard to the causation of the systolic pulsation in the second left intercostal space is to my mind absolutely proved, the murmur heard in that situation in the heart in debility seems to me to be simply a tricuspid systolic murmur propagated upwards by means of the conus arteriosus. It is quite analogous to the murmur produced at the right side of the heart in cases of heart strain, which is undeniably of tricuspid origin. To show that this murmur may have its greatest loudness close to the spot commonly known as the pulmonary area, the following case may be brought forward.

SYLVESTER N., æt. 24, unmarried, strapper in a stable; was admitted to Ward 6 of the Royal Infirmary, on 26th June 1893, with obvious symptoms of alcoholism.

His father, æt. 61, and mother, æt. 60, were in excellent health. Of ten brothers and sisters, only two brothers and one sister were alive, seven having died in their infancy. His social conditions were fairly good, except at times from his own fault.

The patient's previous health had been quite good, but he had been much addicted to drink.

The attack for which he was brought to the hospital began about Christmas, since which time he had been drinking very heavily, and about the middle of May pains in the legs, with some swelling of the ankles, set in.

On admission the patient was found to have great thirst and little appetite; the tongue was furred and shaky; the breath heavy and foul. No other symptoms connected with the alimentary or hæmopoietic systems were present.

There was breathlessness on exertion and swelling of the ankles and legs. The pulse was of low tension, moderate fulness, and perfect regularity. The rate was usually from 80 to 90. There was a well-marked venous pulsation in the neck. On inspection of the præcordia, no impulse could be seen, and the apex beat could only be felt when the patient was placed on his left side.

The deep cardiac dulness extended $2\frac{1}{2}$ in. to the right, and $4\frac{1}{2}$ in. to the left of the middle line at the level of the fourth cartilage. On auscultating the heart, a soft systolic murmur was heard over a great part of the præcordia, with its maximum intensity over the left half of the sternum opposite the attachment of the third cartilage, as is shown in Fig. 3. It was obviously a murmur of tricuspid regurgitation, heard most distinctly over the infundibulum.

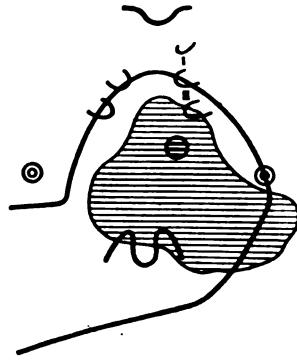


FIG. 3.

No abnormal symptoms, connected with the respiratory or urinary systems, were present. The patient had some insomnia, followed by restless slumber with alarming dreams, and he had a distinct tremor throughout the entire muscular system.

Under appropriate treatment the nervous disturbances passed away, and the patient was transferred to Ward 22, where he speedily lost all the swelling and breathlessness. The

physical signs connected with the heart had in great part disappeared when he was discharged.

In this case there could be no doubt that the murmur described was due to escape at the right auriculo-ventricular orifice, and its localisation throws much light on the question that has been discussed.

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ON LOCAL AND GENERAL IMMUNITY:

AN INVESTIGATION BASED UPON EXPERIMENTAL ERYSIPELAS IN ANIMALS; TOGETHER WITH SOME OBSERVATIONS ON THE MODE OF GROWTH AND LIFE-HISTORY OF THE STREPTOCOCCUS ERYSIPELATIS.

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From the Cambridge Pathological Laboratory.

- I. Introduction.
- II. Methods.
- III. Mode of growth of *Streptococcus erysipelatis*.
- IV. Cause of cessation of growth of the streptococcus in beef-broth.
 - V. On the changes in virulence produced by varying the artificial media.
- VI. Method of increasing the virulence of the streptococcus.
- VII. On the effect of intra-abdominal injections of *Streptococcus erysipelatis*.
 - (a) Disappearance of the micro-organisms from the peritoneal cavity.
 - (b) On metastatic erysipelas.
- VIII. On the immunity conferred upon the abdominal cavity against virulent cultures of *Streptococcus erysipelatis* by previous injections of attenuated virus.
- IX. On the effect of inoculating the ears of rabbits with streptococci—
 - (a) The occasional appearance of relapses and their nature.
- X. Comparing the results of inoculations into parts which had been directly affected by erysipelas with those of similar inoculations into other parts of the same animals.
 - (a) On general immunity, conferred by an attack of cutaneous erysipelas.
 - (b) On local immunity, conferred by cutaneous erysipelas, as shown by the effect of second inoculations of ears which had recently been the seat of erysipelas.
- XI. On the cause of the inflammatory reaction which resulted when ears, locally protected by recent erysipelas, were reinoculated with living streptococci.
- XII. On the production of general immunity by intra-peritoneal injections of attenuated cultures of *Streptococcus erysipelatis*.
- XIII. On the relation of inflammatory reaction to immunity.
- XIV. On the relation of inflammation to recovery from erysipelas.
- XV. On immunity produced by the chemical products of the *Streptococcus erysipelatis*.
- XVI. Conclusions.

I. INTRODUCTION.

WHEN pathogenic micro-organisms have once succeeded in establishing themselves in the body of a living animal, and by growing and multiplying have caused the symptoms of disease, we might naturally expect that they would continue to flourish there until they had destroyed their host; yet many microbic diseases, under favourable circumstances, terminate in recovery. At some period during the course of these affections a change takes place in the body of the patient, which makes it a less favourable place for the microbes to live in than before; they consequently disappear, and recovery ensues. Moreover, for some time afterwards, the patient is found to be less susceptible to the attack of the same microbe. The supposition, that immunity is due to the same change which brought about recovery from the first attack of the disease, has commended itself to many observers. Now, it is well known that, in erysipelas, recovery in the part first affected may commence while the disease is actively spreading elsewhere. Here a local change must be the cause of the local recovery, and it seemed worth while to inquire whether this is followed by any local immunity. It was in order to investigate this point that the present research was undertaken.

But erysipelas in favourable cases terminates before the whole surface of the body has been affected, and consequently this ultimate recovery must be due to some change which affects the whole of the body, and this, on the supposition alluded to above, should, for as long a time as it persists, cause general immunity. That erysipelas confers an immunity of short duration, though overlooked by clinical observers, has been fully demonstrated by Fehleisen, Roger, and others, but none of these observers distinguish between local and general immunity.

Fehleisen, who inoculated patients suffering from cancer with erysipelas, found it impossible to communicate the disease to those who had recently recovered from an attack. The longest period of immunity which he observed was a few months only. More recently Roger has shown that it is possible to immunise rabbits by means of intravenous injections of living broth cultures, or of filtered cultures heated to 110° C. And he points out that, after an attack of cutaneous erysipelas, a second inoculation produces either a mild form of the disease, or merely a small abscess.

Since it is true that an attack of erysipelas is followed by general immunity, it will be impossible to obtain evidence of local immunity, unless the parts which have been directly affected have acquired a protection which is more efficient or of longer duration than that acquired by the rest of the body. We might indeed be able to obtain evidence of local immunity in a part which had recovered before the disease had ceased to spread elsewhere, but, in the animals at our disposal, erysipelas did not extend widely enough to make this possible. We hope to be able to show that this difficulty did not prove a serious

obstacle, but, before describing this part of our work, it will be necessary to say something of the methods employed, and desirable to make some observations upon the life history and mode of growth of the streptococcus.

II. METHODS.

In our experiments we used rabbits, which not only possess the advantage of being susceptible, but also of having large ears, which are very convenient for observing the effects of an inoculation.

Our original cultures were obtained from cases of erysipelas in man; one from a patient suffering from the facial form of the disease, and two others from children in whom the inflammation had spread widely over the surface of the body.

The method of obtaining these cultures was the following:—A part was selected where the disease was actively spreading by a definite margin. This was sterilised by washing successively with soap and water, ether, and perchloride of mercury, 1 in 1000. Absolute alcohol was then applied to remove the mercury, and when the skin was dry a small puncture was made with a sterilised needle. A drop of fluid was then squeezed out, and this was transferred by means of a platinum wire to one or more agar tubes and spread over their surfaces. Some difficulty was experienced in obtaining any fluid, and it was found advisable to make the puncture at a distance of 1 in. from the actual margin where the swelling was greater.

The tubes were incubated at a temperature of 37° C., and minute colonies of streptococci became visible in 1 or 2 days. In the many tubes sown on these occasions, only one foreign colony appeared. We also, on one occasion, made use of Fehleisen's method of snipping off a piece of the affected skin with sterilised scissors, but we found that this was attended with more risk of contamination; due, no doubt, to the fact that it is impossible completely to sterilise the epidermis. And we preferred the puncture method, on account of its simplicity and certainty. Moreover, it causes very little discomfort to the patient, a matter of some importance if it is to be used as a diagnostic test of erysipelas in man. Many new cultures were obtained in the course of our work from the ears of rabbits affected by erysipelas, and in these cases a slight modification of the method just described was employed. A small area of skin was sterilised by the momentary application of a hot glass rod, which caused no appreciable discomfort. A fine capillary glass tube was then pushed into the subcutaneous tissue, and the fluid which entered it was subsequently blown out on to the surface of nutrient agar. With due care this method was found to be quite free from risk of contamination. From these cultures others were obtained on beef-broth containing peptone, solidified blood serum, and nutrient gelatine.

In order to produce erysipelas, liquid or solid, cultures were rubbed

into scratches, made with a sterilised needle, or liquid cultures were injected by means of a hypodermic syringe, but the following method was found to give the most satisfactory result. A small portion of the surface of the skin having been sterilised in the way just described, a puncture $\frac{1}{4}$ in. long was made under the skin with a needle. A straight platinum wire, rubbed over the surface of a culture on a solid medium, was then introduced into the puncture, and as it was withdrawn the ear was held between the finger and thumb in such a way as to cause the streptococci which had adhered to the wire to be left behind. The puncture was then sealed with collodion. Intra-abdominal injections were made by means of a capillary glass pipette, pushed through the abdominal wall, after the skin had been sterilised with a hot rod. This procedure was found to be unattended by risk of wounding the intestine.

The inflammatory changes produced by inoculating the rabbits' ears were recorded in notes and drawings, and comparative measurements of the intensity of the inflammation obtained by observing the changes in the volume of the affected parts. These measurements were made in the following way. A cylindrical vessel, of a size just sufficient to admit the swollen ear, was filled with water, and into it the ear was immersed until the brim was in accurate contact with the animal's head. The ear having been withdrawn, its volume was then estimated by measuring the quantity of water which was required to make up for that which had been displaced. Inasmuch as rabbits' ears, while affected by erysipelas, are very liable to become temporarily congested when interfered with in any way, it was necessary, in order to avoid errors which might arise from this cause, to handle them as little as possible, and to employ cold water for their immersion. For the purpose of comparing the changes in volume of ears which, at the time of inoculation, were of different sizes, it was thought desirable to record, not the volumes actually measured, but the ratios of these to the original volume of the ear. Curves were accordingly drawn, in which the ordinates represent these ratios, and the abscissæ equal intervals of time. When these were compared with the notes taken at the same periods as the measurements, they were found to give an accurate representation of the intensity and rate of progress of the acute stages of the inflammation, but no indication of its subsidence, because considerable thickening in all but mild cases remained for many days after redness and heat had disappeared.

III. ON THE MODE OF GROWTH OF THE STREPTOCOCCUS ERYSIPELATIS.

In beef-broth containing peptone the *Streptococcus erysipelatis* grows rapidly at a temperature of 37° C. At times the resulting growth is scanty, at other times copious. In the former case the liquid remains clear throughout. In the latter, it is turbid at first, but becomes clear after a day or two when the micro-organisms have settled at the bottom of the tube.

The duration of the life of the individual coccus is probably very short, for if a tube be sown in the ordinary manner from a broth culture, which has for 3 or 4 days been free from turbidity, growth but seldom takes place. For this reason it was necessary to transfer our cultures to new tubes every few days. Later, however, we found that it is possible to obtain a growth from an old culture, by taking a considerable quantity of the sediment of microbes and putting it into new broth. When this was done, we had no more difficulty in obtaining a culture from one 90 days, than from others a week or 10 days old. These experiments were repeated on many occasions, always with the same results; and we came to the conclusion that the majority of the cocci die about the end of the first week, but that a few remain alive for a considerable period.

The sediment, which collected at the bottom of a flask when active growth ceased, usually diffused itself readily throughout the liquid on shaking, but it was sometimes collected into flocculent masses, which did not readily break up.

The microscope showed that individual cocci varied much in size. Large and small beads were seen in the same chain; and chains—some composed of large and some of small beads—were often present in the same flask. Moreover, in some cultures the cocci were generally of a larger size than in others, and it was in these cultures that the sediment collected into flocculent masses.

Von Lingelsheim,¹ who has recently studied the mode of growth of streptococci, mentions this formation of flocculi. He found that there was a greater tendency to their formation in cultures sown directly from the animal body than in those sown from cultures which had been kept for a long time on artificial media; and he looked upon their presence as a favourable indication of vitality and virulence. On the other hand, in our experience the formation of these flocculi occurred only in cultures which had been grown for many generations in beef-broth, and which possessed little or no pathogenic power.

In broth, without the addition of peptone, there was very little growth.

On agar the colonies varied much in size. As is the case with many other microbes, they remained small when thickly clustered together, and grew larger when situated at a greater distance from their neighbours; they seldom, however, reached the size of a small pin's head. Occasionally all the colonies of one tube were of a larger size, and this was the case when a film of blood containing cocci was spread over the surface of the nutrient medium. They then absorbed the pigment from the blood immediately around them, and appeared surrounded by a colourless halo. In tubes which were fairly dry the colonies were sharply defined and discrete, but on moist agar their outlines were irregular, and they tended to run together and form a uniform film on

¹ *Ztschr. f. Hyg.*, Leipzig, 1891, bd. x. s. 329.

the surface. No difference was found in the virulence of cultures composed of large as compared with those composed of small colonies, but the growths on dry tubes appeared to be more powerful than those on moist.

IV. ON THE CAUSE OF THE CESSATION OF THE GROWTH OF STREPTOCOCCUS ERYSIPELATIS IN BEEF-BROTH.

In order to find out why the streptococcus ceases to grow in broth at the end of the first few days, we undertook some experiments similar to those made by Pasteur¹ on chicken cholera many years ago. The clear fluid of an old culture was drawn up into a pipette and put into sterile tubes. These, together with control tubes of fresh broth, were then sown with various micro-organisms, including the streptococcus. In none of the latter did any growth appear, but *Bacillus anthracis* and other peptonising microbes flourished in the fluid. This led us to add 1 per cent. of peptone in concentrated solution to the old broth, with the result that it became capable of supporting as copious a growth of the streptococci as appeared in the control tubes.

It appears from this that the culture ceases to grow because its supply of nutriment is exhausted. In support of this view we are able to bring forward some evidence that there is no bactericidal body in the broth of an old culture which is capable of preventing further growth.

Buchner found that the bactericidal property of serum is destroyed by exposure to a temperature of 50° C. for one hour.² And it seemed probable that if any similar substance existed in the fluid of our old cultures it would be destroyed by heat. We therefore heated this fluid in the autoclave to 110° C. for 15 minutes, and again sowed it with streptococci. No growth appeared. In other tubes of the same material, which had been exposed to a temperature of 130° C. for 1 hour, a trace of growth did indeed develop after they had been sown. This we attributed, not to the destruction of any bactericidal body, which would probably have taken place at the lower temperature, but to the formation of peptone out of some albuminous body (possibly alkali albumen) in the broth. Further evidence of the absence of any bactericidal substance from the fluid of an old culture was obtained by evaporating it, *in vacuo*, to small bulk, and adding enough fresh broth to restore its original volume. In this mixture the streptococcus grew as freely as in the control tubes sown at the same time.

From these results we conclude that the cessation of growth of a culture of streptococci in peptone broth is due solely to the exhaustion of medium; an opinion identical with that arrived at by Pasteur as the result of his experiments on chicken cholera.

¹ *Compt. rend. Acad. d. sc.*, Paris, 1880.

² *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, bd. v.

V. ON THE CHANGES IN VIRULENCE PRODUCED BY VARYING THE ARTIFICIAL CULTURE MEDIA.

In our experiments on rabbits we first made use of broth cultures, because they were more convenient for injection.¹ When liquid cultures were injected under the skin of the ears, they were found to be incapable of causing erysipelas; even 10 drops produced only a slight transient inflammation or local suppuration.²

Considerable variation was observed in the virulence of different broth cultures when introduced into the peritoneal cavity. For instance, one animal died 24 hours after receiving 1 c.c., another in the same time after 2 c.c., and a third 18 hours after 5 c.c., but this was quite exceptional. In the majority of cases it required 10 c.c. to kill an animal, and recovery sometimes occurred after the injection of 30 c.c. of broth culture.

When broth had been sown with streptococci, taken directly from the body of an animal, it was more virulent than that which had been sown from another broth culture. Observations on the different degrees of virulence of cultures of various ages gave conflicting results. Old growths were in some instances harmless, in others powerful. When the micro-organisms were transferred directly from the body of an animal to agar, or solidified serum, their virulence was not appreciably diminished, and we did not observe any attenuation in cultures grown for several generations on solid media. It was noticed also that cultures increased in virulence when transferred from beef-broth to agar, so that they would then produce erysipelas. We had not, however, discovered these facts at the time of our earlier experiments, and were obliged to increase the virulence of broth cultures in other ways.

VI. METHOD OF INCREASING THE VIRULENCE OF THE STREPTOCOCCUS.

We attempted to increase the virulence of a broth culture of streptococci, first by cultivating them in rabbit's serum. In this medium they grew well, but their pathogenic power was not increased. We next grew them in tubes of beef-broth, from which all oxygen had been expelled, but without success. We succeeded better when we grew them in the abdominal cavities of several rabbits in succession. A large injection of a beef-broth culture having resulted in the death of the first animal, the peritoneal exudation containing cocci was taken up in a pipette and injected into the abdomen of a second rabbit. When the second

¹ Unless otherwise stated, the broth cultures used for injection were 2 or 3 days old, and their vitality was proved by re-sowing.

² Since this was written we have obtained from a man suffering from erysipelas, a culture of streptococci, which, when cultivated in beef-broth, even for two or three generations, was capable of causing erysipelas in rabbits.

animal died its peritoneal fluid was injected into a third, and so on, until the culture had passed through 3 or 4 rabbits. It was then found to have reached such a degree of virulence that 2 or 3 drops of the peritoneal exudation, put into the abdomen of another animal, produced death in about 12 hours or even less; and when the same material was rubbed into little scratches in a rabbit's ear, in the manner in which vaccine lymph is usually applied, it produced severe erysipelas.

Nevertheless, if sown on broth, its virulence immediately diminished, and it was necessary to inject into the abdomen 10 c.c. of such a culture to produce death.

VII. THE EFFECT OF INTRA-ABDOMINAL INJECTIONS OF STREPTOCOCCUS ERYSIPELATIS.

Shortly after intra-abdominal injections of 10 c.c. or more of living broth cultures, the animals began to show symptoms of illness. In about an hour they had become less active than their fellows and refused food; a little later they might be seen crouching in the corner of the cage, rocking to and fro with each respiratory movement. The temperature fell immediately after the injection, sometimes as much as 2° or 3° F. In the course of a few hours it rose above the normal, and in more than one instance it reached 106° F. on the second day.¹ In fatal cases it fell considerably, before death, which occurred in from 4 to 45 hours from the time of injection. Diarrhoea was commonly observed in those rabbits which survived for more than 12 hours.

Post-mortem examination revealed the presence of more or less inflammatory oedema in the subcutaneous tissues about the puncture. Within the abdomen the signs of peritonitis were comparatively slight, a few c.c. of turbid fluid, sometimes blood stained, were found. A little lymph was often seen gluing the large intestine to the parietal peritoneum, near the track of the pipette, and the same material was sometimes found about the edges of the spleen. The omentum which, as a rule, appeared to have suffered more severely, was commonly found drawn up, oedematous, and reddened by hæmorrhages. The intestines were distended with watery material. The lungs were often congested, and the pleural and pericardial cavities contained an excess of fluid.

(a) *On the disappearance of the streptococci from the peritoneal cavity.*—In many cases the peritoneal exudation contained multitudes of free streptococci; in others, they were found only in the interior of the cells; and in a few none could be seen. Growth commonly, but not invariably, occurred when the fluid was sown on nutrient media.

¹ The normal temperature of a rabbit is 102°–103° F.

We observed that in those animals which succumbed quickest, free cocci were very numerous in the peritoneal exudation; and in those which survived longest they were either absent, or contained within phagocytes. In very young rabbits they disappeared earlier than in older ones. To this rule there were frequent exceptions, and we must rely on the accompanying table to show upon what evidence it rests.

TABLE I.

CLASS I.—IN WHICH BEEF-BROTH CULTURES WERE USED FOR INJECTION.			
Rabbits 8–12 weeks old. Weight 800–1100 grms.	Dose.	Time of Death.	Streptococci in the Peritoneal Cavity.
A	18 c.c.	4½ hours	Very numerous.
B	45 c.c.	5 "	Do.
C	18 c.c.	8 "	Do.
D	3 c.c.	12 "	Numerous.
E	5 c.c.	15½ "	Do.
F	38 c.c.	18 "	Do.
G	5 c.c.	18 "	None observed, not sown.
H	15 c.c.	19 "	Very numerous.
I	13 c.c.	20 "	Do.
J	15 c.c.	20 "	Not examined.
K	11½ c.c.	22 "	Numerous.
L	14 c.c.	23 "	Do.
M	1 c.c.	24 "	None observed, not sown.
N	(20 days old.) 2 c.c.	24 "	Very numerous.
O	10 c.c.	30 "	Few, many in cells.
P	5 c.c.	36 "	Numerous.
Rabbits 4–5 weeks old.			
Q	20 c.c.	4 "	None observed, but growth on sowing.
R	(43 days old.) 3 c.c.	9 "	None observed, not sown.
S	9½ c.c.	42 "	Do. but growth on sowing.

CLASS II.—IN WHICH PERITONEAL EXUDATION CONTAINING COCCI WAS MIXED WITH BEEF-BROTH, INCUBATED FOR A FEW HOURS AND USED FOR INJECTION.			
Rabbits 8–12 weeks old.			
A	6 c.c.	7 hours	Very numerous.
B	6 c.c.	8 "	Do.
C	2½ c.c.	8 "	Not examined.
D	12 c.c.	11 "	Do.
E	4½ c.c.	16 "	Present.
F	5 c.c.	16 "	Do.
G	1 c.c.	36 "	Not examined.
H	8 c.c.	44 "	Present in cells only.
Rabbit 4–5 weeks old.			
I	½ c.c.	28 "	Do. do. growth on sowing.

TABLE I.—*continued.*

CLASS III.—IN WHICH PERITONEAL EXUDATION, DIRECT FROM ANIMALS WHICH HAD RECENTLY DIED OF AN INTRA-PERITONEAL INJECTION, WAS USED.			
Rabbits 8-12 weeks old. Weight 800-1100 grms.	Dose.	Time of Death.	Streptococci in the Peritoneal Cavity.
A	1 c.c.	5½ hours	Very numerous.
B	(?)	7 "	Do.
C	4 drops	12 "	Do.
D	3 c.c.	12 "	Not examined.
E	1 c.c.	13 "	Do.
F	2 c.c.	15 "	Very numerous.
G	3 c.c.	15 "	Do.
H	½ c.c.	18 "	Present in cells only.
I	5 c.c.	54 "	Not examined.
J	4 drops	4 days	Do.
Rabbits 4-5 weeks old.			
K	3 drops	10½ hours	Present.
L	3 drops	17 "	Do.
M	Grown from a serum tube	7 days	Few in cells only, growth on sowing.

Streptococci were often found in the pericardial and pleural fluid.

In the blood, after death, they were commonly present. In 3 only, out of 13 cases in which we sowed the blood on nutrient media, did we observe no growth. The fact that 20 to 30 colonies appeared on the surface of agar tubes sown with a single drop of blood gave a rough indication of their relative numbers.

In those cases, in which no streptococci could be demonstrated by culture experiments to be present in the peritoneal exudation, they were found in the blood, and it was evident that even in many of those animals which died of septicæmia after an abdominal injection, the streptococci had more or less completely disappeared from the peritoneal cavity.

In order to find out how quickly they disappear from this situation in non-fatal cases, 2 rabbits which appeared about to recover were killed. In the first, which had received 5 c.c. of a broth culture (2 days old) 30 hours before, only one chain of free streptococci was found after prolonged search, but many cocci were contained in cells, and broth sown with this fluid grew a good culture. The second rabbit having shown no signs of illness after an injection of 6 c.c. of an anærobic broth culture (2 days old), received, next day, 10 c.c. of a similar material, 1 day old, swarming with streptococci. When killed 5½ hours later, not only could no streptococci be seen either free or in cells, but no growth developed in broth sown with the abdominal fluid.

(b) *On metastatic erysipelas.*—Out of 41 animals which had been inoculated in the peritoneal cavity for the first time, with cultures of streptococci, 3 developed erysipelas in the ear a few days later.

In the first case 28 c.c. of a beef-broth culture were injected into the peritoneal cavity of a rabbit weighing 1160 grms. A considerable quantity of the fluid was forced out of the abdomen into the subcutaneous tissue, and a fluctuating swelling was thus produced. This was rapidly absorbed, but a ring of inflammation appeared around the place where the swelling had been, and spread until it was $1\frac{1}{2}$ in. wide; after this no further extension occurred. On the seventh day erysipelas appeared in the right ear, and increased in severity till the death of the animal on the ninth day.

In the second case, a rabbit weighing 840 grms. received 16 c.c. of a beef-broth culture. On the following day it appeared quite well. Twenty-four hours later it was again injected with 15 c.c. of a more virulent broth culture. On the following day there was an erysipelatous patch about $1\frac{1}{2}$ in. in diameter about the seat of inoculation, and 12 hours later erysipelas appeared in the right ear. It ran a mild course, with slight disturbance of general health, and the animal recovered.

The third instance of metastasis occurred in a rabbit weighing 720 grms., which had had injected into the abdominal cavity 6 c.c. of a broth culture, sown direct from the peritoneal fluid of an animal, and therefore more virulent than that used in the two cases just described. For the next 4 days it remained quiet and ate little. On the eighth day erysipelas appeared in the right ear and spread over the head; on the ninth day it had reached the opposite ear, and the animal died the same evening. Streptococci were grown from fluid taken from the spreading margin on the left ear during life, and from the blood after death.

VIII. ON THE IMMUNITY CONFERRED UPON THE ABDOMINAL CAVITY AGAINST VIRULENT CULTURES OF *STREPTOCOCCUS ERYSIPELATIS* BY PREVIOUS INJECTIONS OF ATTENUATED VIRUS.

In order to find out how far a previous injection of a weak culture of streptococci increases the resisting power of the peritoneum, we injected into the abdominal cavity of a number of rabbits, which had recovered from their first injection, a second dose of more virulent cultures; some of the animals received 3 or even 4 injections; control animals were used in all cases.

The results of these experiments show that the previous injections had considerably increased the resistance of the peritoneum. In 15 out of 18 experiments, the animals, which had received a previous injection, recovered from a second, third, or fourth injection of most virulent material, in quantities as large, and in some instances considerably larger, than those which killed the controls. On one occasion the dose was too weak to kill either animal. In the three instances in which the animals died of the second or third injection, we must suppose

TABLE II.—*Showing the Immunity conferred upon the Abdominal Cavity by Intra-Peritoneal Injections of Cultures of Streptococcus erysipellatis.*

FIRST INJECTION.				SECOND INJECTION.				THIRD INJECTION.				FOURTH INJECTION.			
Animal.	Weight in Grammes.	Dose.	Result.	Interval.	Weight.	Dose.	Result.	Interval.	Weight.	Dose.	Result.	Interval.	Weight.	Dose.	Result.
I.	800	20 c.c. B (7 age)	Recovery	1 day No control	?	45 c.c. B	Recovery	1 day Control A Control B	?	30 c.c. B 10 c.c. B 45 c.c. B	Recovery Death 30 hours	8 days Control	800? 400	4 c.c. P 15 c.c. P	Recovery Death 10 hours
II.	840	15 c.c. B (3 days old)	Recovery	5 days Control	800 1100	6 c.c. P+B 6 c.c.	Recovery Death, 7 hours	10 days Control	850 875	54 c.c. P+B 24 c.c.	Both found dead next morning	Dose probably too large			
III.	1175	30 c.c. B (3 days old)	Recovery	5 days	1025	8 c.c.	Recovery								
IV.	1040	19 c.c. (48 days old)	Recovery	5 days Control	1040 1085	8 c.c. P+B 8 c.c.	Recovery Death, 40 hours								
V.	840	16 c.c. B (5 days old)	Recovery	5 days Control	885 575	15 c.c. P+B 94 c.c.	Recovery Death, 41 hours	7 days Control	920 940	124 c.c. B 124 c.c.	Recovery 2nd day Recovery 3rd day Dose not large enough	8 days Control	880 870	16 c.c. B 14 c.c.	Recovery Death 22 hours
VI.	515	One agar culture	Recovery	5 days Control	510 480	5 c.c. B 5 c.c.	Recovery Death, 24 hours								
VII.	?	20 c.c. B (7 age)	Recovery	6 days Control	425 925	4 c.c. P 4 c.c.	Recovery Death, 17 hours								
VIII.	880	10 c.c. B (12 days old)	Recovery	6 days Control	850 940	1 c.c. P+B 1 c.c.	Death, 18 hours Death, 28 hours	This rabbit the peritoneal cavity was full of cysticercal		had severe peritonitis of liver, and cavity was full of cysticercal					
IX.	940	12 c.c. B (1 day old)	Recovery	9 days Control	700 750	8 c.c. P+B 8 c.c.	Recovery Death, 9 hours								
X.	925	9 c.c. B (2 days old)	Recovery	14 days Control	1080 1280	6 c.c. P+B 6 c.c.	Recovery Death, 40 hours								
XI.	830	30 c.c. B (3 days old)	Recovery	15 days Control	835 895	54 c.c. P+B 44 c.c.	Both found dead next morning	Dose probably too large							
XII.	1320	94 c.c. B (7 age)	Recovery	15 days Control	1300 1000	Similar dose of same serum culture	Recovery Death, 7 days								
XIII.	1010	Soluble products	Recovery	8 days Control	510 480	5 c.c. B 5 c.c.	Recovery Death, 24 hours								

M. B.—B=Beef-broth culture. P=Peritoneal exudation fluid containing cocci, from an animal which had just died of an abdominal infection of a living culture. P+B=Mixture of peritoneal exudation fluid with a little beef-broth inoculated for a few hours at 37° C.

that the dose was too large, and that, although the immunity conferred on the peritoneum by previous injections is considerable, yet it is not complete: the animals may die if the virus be very strong.

That this increased resistance of the peritoneum to the streptococcus is a specific immunity to that microbe and no other we do not assert. Bearing in mind that Klein¹ was able to produce immunity against injections of the cholera spirillum into the abdominal cavities of guinea-pigs by previous injections in the same situation of various other microbes, we are inclined to think that the inflammation rendered the peritoneum more resistant to bacterial invasion in general; but on this point we have no evidence. It is interesting to notice that clinical observations on man accord with these views. Treves² points out that abdominal operations on persons who have previously suffered from peritonitis are attended with less danger of septic complications than like operations on the healthy peritoneum.

The question, whether an injection of the streptococcus into the peritoneal cavity confers immunity on other parts of the body than those directly affected by it, will be more conveniently discussed when we have described the effect of inoculating the ears of fresh rabbits.

IX. ON THE EFFECT OF INOCULATING THE EARS OF RABBITS WITH STREPTOCOCCUS ERYSIPELATIS.

We have already mentioned that when broth cultures were injected under the skin of the ear, transient redness, and sometimes the formation of a minute quantity of pus, were the only consequences which followed. The more virulent material which we found necessary to use, in order to produce erysipelas, was either the peritoneal exudation of a rabbit which had died of an abdominal injection, or the growth from the surface of an agar tube. Between these two materials there is an important difference. The former doubtless contains, in addition to the microbes, the extra-cellular products of their vital activity; while the latter contains presumably nothing but the microbes themselves. A corresponding difference, in the mode and rate of progress of the inflammation which resulted from the injection of these two materials, was also observed.

The peritoneal exudation was either injected, in quantities of one to three drops, under the skin of the ear, or rubbed into scratches. The agar growths were, in our earlier experiments, suspended in a minute quantity of fresh broth, and used in the same manner; in our later experiments a platinum wire, rubbed over the surface of the culture, was inserted into a puncture made with a sterilised needle.

When the peritoneal exudation was used, the following appearances were observed. After 3 or 4 hours the skin about the seat of inocula-

¹ *Brit. Med. Journ.* 1893, p. 632.

² *Ibid.* February 3rd, 1894.

tion was found to be warmer than elsewhere and slightly red and swollen. These appearances were not sharply limited, but gradually faded towards the healthy parts. In about 12 hours there was slight oedema, and the vessels of the ear appeared blurred when seen by transmitted light. The swelling gradually increased, and the ear became hot, tense, and somewhat greasy. The inflammation reached its maximum, in severe cases, on the eighth or ninth day; in mild cases it began to diminish a few days earlier. The subsidence of the active signs of inflammation was rapid; heat and tension quickly disappeared, and the surface, no longer moist and greasy, became dry and scaly, but the oedema lasted for a considerable time. After this had gone the ears remained, in all but very mild cases, much thickened for several weeks, and the hair fell out, so that sometimes, after severe erysipelas, the ear became quite bare.

We were disappointed at the absence of the definite spreading margin, so characteristic of erysipelas in man. This we supposed to be due to the action of poisonous bacterial products present in the peritoneal exudation injected, which, by rapidly diffusing and causing inflammation, had obscured the effects of the more gradual advance of the microbe.

This supposition received support on three occasions, when the erysipelas spread to the opposite ear. In this situation, to which it was unlikely that the poisonous products had travelled in a direction contrary to the natural lymph stream, the inflammation spread by a definite margin, in the oedema fluid of which we found streptococci.

When erysipelas was produced by the inoculation of cultures on solid media, the inflammation spread by a similar definite margin from the commencement. The first sign was a small pale and slightly raised oedematous area extending a few mm. on each side of the puncture. In most cases this was first seen about 6 hours after the inoculation, and in 18 hours this patch had reached the size of a shilling, its yellowish-white colour contrasting with the more translucent bluish appearance of the surrounding parts, and the raised edge clearly defining its limits. Moreover, the orifices of the glands became very obvious, and between their depressions the skin was thrown into delicate wrinkles, giving, on a small scale, the appearance of leather made of pigskin. By transmitted light the central part of the patch appeared red, but the redness always stopped short of the margin. The vessels of the affected part were obscured; in the rest of the ear they were clearly seen; as a rule they were but little injected, unless the ear had been recently handled. The main central vessels, however, were slightly blurred, some lymphangitis having occurred along their course, and the auricular and submaxillary glands were enlarged. The margin of the inflammation gradually advanced over the ear, the whole of which became affected on the third day. About this time the ear drooped. The subsequent course was the same as in those inoculated with peritoneal exudation. A little pus was often

seen about the seat of the puncture, and in one instance small abscesses appeared in other parts of an ear which was unusually severely affected. In three cases the onset was delayed until the fourth or fifth day, and on more than one occasion erysipelas began at the root and spread towards the seat of inoculation.

On the Occasional Appearance of Relapses and their Nature.—In four instances inflammation reappeared in parts which were returning to their normal condition. This phenomenon is in apparent contradiction to the theory that erysipelas confers local immunity, and it is therefore desirable to describe these cases in detail, and to inquire whether this inflammation be true erysipelas.

The first instance of relapse occurred in a rabbit which had been inoculated in the ear with peritoneal exudation. On the following day a little inflammation appeared about the seat of inoculation, but this faded in a few hours, and the ear remained normal until the fourth day. The ordinary appearances of erysipelas were then observed to be present over the posterior third of the ear from root to tip. The inflammation then advanced towards the anterior border, and gradually faded in the part first affected. On the eighth day the anterior third was red and swollen, and the posterior two-thirds were almost normal in appearance. On the ninth, however, the whole ear was found to be hot and swollen, and on the tenth the animal died.

The remaining instances of relapse occurred when the signs of active inflammation had disappeared from the whole ear. On the tenth day in two cases, and in the other on the seventeenth, after the first inoculation, the parts which had just recovered from erysipelas again became hot, red and swollen; this recurrent inflammation appeared simultaneously in the whole ear, and rapidly subsided in about 48 hours. Two of the animals recovered, the other died about 5 days later, probably from some other cause, for no streptococci could be grown from the blood. Similar relapses have been observed in cases of erysipelas in man. In these the inflammation reappears simultaneously over a considerable area, no advancing margin is observed, and it lasts but a short time.

We shall have to point out later that when the products of the streptococcus are injected into an ear, which has recently recovered from erysipelas, a somewhat severe though transient inflammation appears almost simultaneously over the whole ear, differing markedly from the milder inflammation produced by the introduction of these poisons into other parts; and we would suggest that the relapses which we observed in our rabbits, and which occur occasionally in man, are not true erysipelas, that no second invasion of the part by micro-organisms takes place, but that the appearances are caused by diffusion of the products of the microbes growing in a part recently invaded; some cause, such as a general disturbance of health, due to some intercurrent disorder, having led either to a weakening of the resistance of the patient,

and to the consequent increase in the quantity of poisons produced, or to a failure to neutralise these poisons *in situ*.

It is to be regretted that we did not examine the œdema fluid of the ears by culture experiments during these recurrent inflammations, but at that time we did not recognise the importance of doing so.

Primary erysipelas in the ear was fatal in 19 out of 35 cases. In two of these death was probably due to other causes. Omitting the latter the time of its occurrence was between the third and tenth day after the inoculation.

Streptococci were found in the blood of 7 out of 11 cases examined after death by culture experiments. Our experience in these respects differs from that of Fehleisen,¹ who produced in rabbits erysipelas which never resulted in death; but it agrees with that of Roger,² who found that erysipelas in these animals was often fatal, and that it was possible to grow streptococci from their blood after death.

X. COMPARING THE RESULTS OF INOCULATION INTO PARTS WHICH HAD BEEN DIRECTLY AFFECTED BY ERYSIPELAS WITH THOSE OF SIMILAR INOCULATIONS INTO OTHER PARTS OF THE SAME ANIMAL

Having succeeded in causing erysipelas in rabbits' ears, we proceeded to determine whether any local or general immunity had been conferred. We accordingly inoculated, at the same time, both ears of animals which had recovered from an attack in one ear, and observed the differences in the inflammations which resulted.

Most of the animals used in these experiments had recently recovered from their first attack, and the thickening which it had caused in their ears had not yet disappeared. This thickening varied considerably in different cases, and depended as much on the severity of the first attack of erysipelas as on the shortness of the interval between the inoculations.

Control animals were inoculated in nearly all cases, and great care was taken that in each experiment both ears of the animal whose immunity was to be tested, and also that of the control, should receive exactly the same quantity of the same culture.

The table on pp. 55, 56, 57 gives the results of these experiments.

We must first inquire what amount of general immunity had been conferred by the previous attack of erysipelas, before we can draw any inference about local immunity. We will, therefore, proceed to describe the results of inoculations into ears which had not been directly affected by the previous inflammation. We may here state, for convenience of description, that in all cases it was the right ear which had been the seat of the primary erysipelas.

(a.) *On general immunity conferred by an attack of cutaneous erysipelas.*

¹ "Ætiologie des Erysipelas," 1883.

² Roger, *Rev. de méd.*, Paris, 1892.

—The effects of inoculating the left ears of rabbits which had recently recovered from erysipelas in the right, varied considerably in different instances (Table III.). We have, accordingly, arranged these experiments in three classes, according to the intensity of the inflammation which resulted. In 6 animals, placed in Class I., erysipelas developed and ended fatally. Streptococci were found in the oedema fluid taken from the ears at some distance from the seat of inoculation, and also in the blood after death. In 4 animals, placed in Class II., the left ears became inflamed, and the appearances at first were like those of commencing erysipelas, but in 2 or 3 days the inflammation completely disappeared. In 4 animals, placed in Class III., nothing but a little redness, and in 2 of them a drop or two of pus resulted from the second inoculation.

In 13 of the 15 control animals, erysipelas developed, ran the usual course, and ended fatally in 6.

TABLE III.—*Showing the Effect of Inoculating with Streptococcus erysipelatis both Ears of Rabbits, which had recovered from Erysipelas in the Right Ear.*

CLASS I.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND ERYSIPELAS IN THE LEFT EAR ; SHOWING LOCAL BUT NO GENERAL IMMUNITY.					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
A.	These two rabbits were inoculated with equal quantities of the same culture. Both developed erysipelas of a mild type, which ran a similar course in each, and subsided on the fifth day.	After 6 days they were again inoculated with equal quantities of same culture. One in the right, and the other in the left ear.	Not inoculated.	Erysipelas, death eighth day, cocci in blood.	Did not take.
			Inflammatory reaction (3 days).	Not inoculated.	
B.	Same as the above A', but subsiding on the sixth day.	Same as the above, but the interval was 12 days.	Not inoculated.	Erysipelas, death sixth day, cocci in blood.	Erysipelas, recovery sixth day.
			Inflammatory reaction (mild). Death, however, occurred on the fourth day from another disorder. No cocci were found in blood.	Not inoculated.	

TABLE III.—*continued.*

CLASS I.— <i>continued.</i>					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
C.	Erysipelas (4-day course).	9 days.	Inflammatory reaction.	Erysipelas, death fifth day.	Erysipelas, death fifth day.
D.	Erysipelas (6-day course).	10 days.	Inflammatory reaction.	Erysipelas, death fourth day, cocci in blood.	Erysipelas, death eighth day, cocci in blood.
E.	Erysipelas (6-day course).	12 days.	Inflammatory reaction (3 days).	Erysipelas, death seventh day.	Erysipelas in 2 controls, one died.
F.	Erysipelas (8-day course) with relapse on the fifteenth day.	17 days.	Inflammatory reaction (mild).	Erysipelas, death third day.	Erysipelas, 10 days, recovery.
CLASS II.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND COMMENCING BUT ABORTIVE ERYSIPELAS IN THE LEFT EAR; SHOWING COMPLETE LOCAL AND LESS GENERAL IMMUNITY.					
A.	Erysipelas (7-day course).	9 days.	Inflammatory reaction (3 days).	Inflammatory reaction commencing more slowly than in the right, ending fifth day. Suppuration.	Erysipelas 6 days (recovery).
B.	Erysipelas (9-day course).	15 days.	Inflammatory reaction (2 days).	Began like erysipelas, aborted third day and suppured.	Erysipelas (severe) in 2 controls, 1 died.
C.	Erysipelas (12-day course).	17 days.	Inflammatory reaction (2 days).	Inflammation like erysipelas, ending fourth day.	Erysipelas (12-day course).
D.	Erysipelas.	30 days.	Inflammatory reaction (3 days).	Inflammation more severe than in the right, ending third day.	Inflammatory reaction (3 days).

TABLE III.—*continued.*

CLASS III.—IN WHICH THE SECOND INOCULATION RESULTED IN AN INFLAMMATORY REACTION IN THE RIGHT, AND LESS REACTION IN THE LEFT EAR; SHOWING COMPLETE LOCAL AND GENERAL IMMUNITY.					
FIRST INOCULATION.			SECOND INOCULATION.		
	Right Ear.	Interval.	Right Ear.	Left Ear.	Control.
A.	Erysipelas (10-day course).	12 days.	Inflammatory reaction (severe, ending fifth day.	Local redness and suppuration.	Erysipelas, death fifth day.
B.	Erysipelas (8-day course).	13 days.	Inflammatory reaction (2 days).	Local redness and suppuration.	No control.
C.	Erysipelas (6-day course).	22 days.	Inflammatory reaction.	Local redness.	No control.
D.	Erysipelas (very severe and prolonged).	37 days.	Inflammatory reaction (3 days).	Local redness.	No control.
CLASS IV.—IN WHICH THE SECOND INOCULATION RESULTED IN ERYSIPELAS IN BOTH EARS, SHOWING ABSENCE OF LOCAL AND GENERAL IMMUNITY, THE INTERVAL BETWEEN THE TWO INOCULATIONS BEING TOO LONG.					
A.	Erysipelas (mild) ending fifth day. A second inoculation produced an inflammatory reaction ending third day.	22 days from the first inoculation.	Erysipelas commencing sixth day, well developed on the seventh day.	Erysipelas began on the second day, no progress till sixth day, well developed on the seventh day.	Erysipelas, death eighth day.
B.	Erysipelas (severe).	118 days.	Erysipelas (severe) subsiding slowly from seventh day.	No inoculation.	No control.
C.	Erysipelas (severe).	135 days.	Erysipelas subsiding seventh day.	No inoculation.	Erysipelas 5 days (recovery).

(b.) *On local immunity conferred by cutaneous erysipelas, as shown by the effect of second inoculations of ears which had recently been the seat of erysipelas.*—The results of second inoculations of the ears *directly affected by the previous erysipelas* were constant. We cannot record a single instance of erysipelas appearing in those which had *recently* recovered from the first attack. In two instances, where the interval between the first and second inoculations was over 100 days, erysipelas did develop, and ran

the usual course; and in one other case in which the primary erysipelas had been very mild, and had subsided on the fifth day, erysipelas was produced after an interval of 22 days. In these 3 animals (Table III. Class IV.) we may suppose that the period of immunity was over, and it is important to note that at the time of the second inoculation no thickening remained.

In all the other cases the only result was a transient inflammatory reaction, lasting 2 or 3 days, and we could get no evidence of any invasion of these ears by streptococci, from an examination of agar

tubes sown with the œdema fluid of their ears, as we were able to do in cases of true erysipelas.

We may sum up the results of these experiments by saying that the first attack of cutaneous erysipelas had conferred a distinct general immunity upon more than one-half of the animals experimented on, but that the rest proved as susceptible as the control animals. On the other hand, an absolute local immunity had been conferred upon the parts directly affected by the first attack, unless the interval had been long enough to permit of the entire disappearance of all inflammatory thickening.

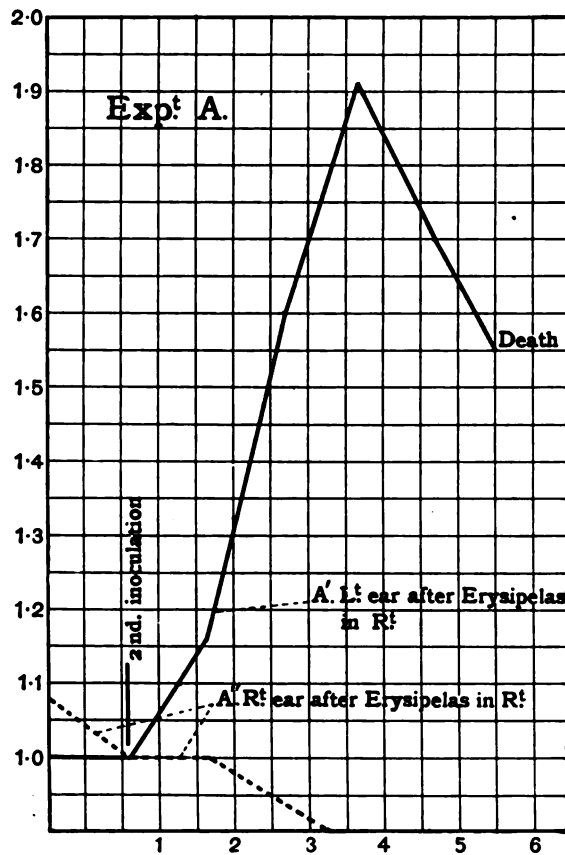


FIG. 1.

The reason for the variation in the amount of general immunity conferred did not very clearly appear. But it seems reasonable to suppose that it depended, partly at least, upon differences in the severity of the first attack, and it will be noticed in the tables that in those animals which proved to have no general immunity (Class I.), the primary erysipelas had run a shorter and less severe course than in those which proved to be better protected (Classes II. and III.) We must also mention that 2 out of the 6 rabbits which showed no general

immunity, were, at the time of the second inoculation, suffering from a disorder, the chief symptom of which was a profuse discharge of saliva and nasal secretion; and it is possible that the disturbance of general health diminished their resistance to the streptococcus.

The accompanying figures give some curves, in which are compared the relative intensity and duration of the inflammation which resulted from a second inoculation, in the ear locally protected by a previous erysipelas, and in the other.

The abscissæ represent days, the ordinates the ratios of the volumes actually measured, in the manner already described (p. 42), to the volume of the ear immedi-

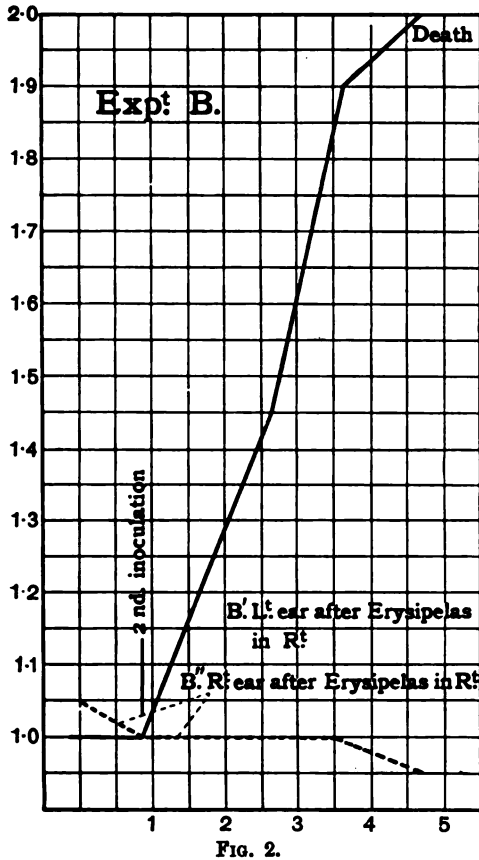


FIG. 2.

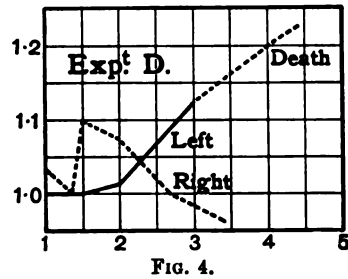


FIG. 4.

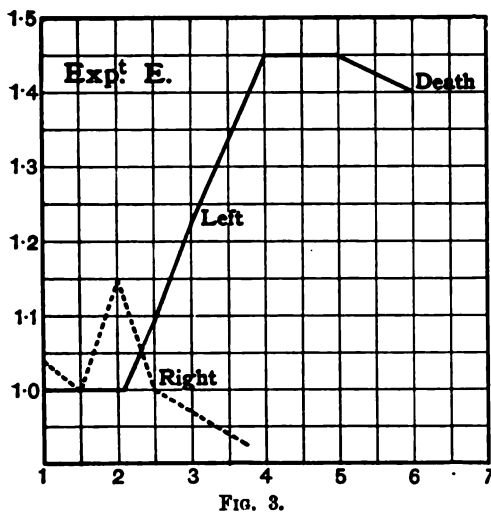


FIG. 3.

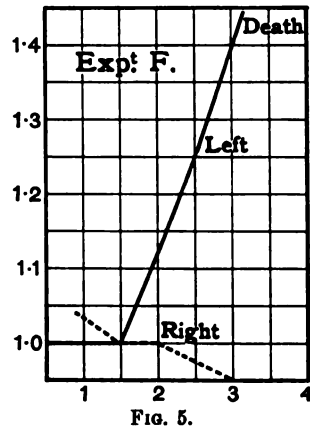


FIG. 5.

ately before the second inoculation. For example, if at the time of the second inoculation the volume of an ear was 10 c.c., and on the fourth day it had increased to 20 c.c., the ratio $20:10=2$ will indicate that the ear has become doubled in size, as the result of the inoculation. In this way the proportional increase in volume of the two ears is compared.

It must be remembered that, at the time of the second inoculation, the ears previously affected were still swollen and steadily diminishing in volume. For such ears, then, a horizontal curve of volume indicates, not that the second inoculation was without effect, but that its effect was to check the diminution which was taking place. Moreover, the method of measuring volumes was not sufficiently delicate to give any evidence of slight degrees of inflammation, which were made quite obvious to the hand and eye by increased heat and redness.

Figs. 1, 2, 3, 4, and 5 are from experiments A, B, D, E, F (Table III. Class I. pp. 55 and 56). They illustrate the fact that these rabbits, which had recently recovered from erysipelas in the right ear, were completely immune to inoculations of the streptococci into these ears, but that they succumbed to erysipelas when similarly inoculated in the opposite ears.

Figs. 3 and 4 show in addition the transient inflammatory reaction which occurred in the protected ears. In the other experiments the reaction in the protected ears was too slight to cause any appreciable increase in volume.

XI. ON THE CAUSE OF THE INFLAMMATORY REACTION WHICH RESULTED WHEN EARS LOCALLY PROTECTED BY RECENT ERYSIPELAS WERE REINOCULATED WITH LIVING STREPTOCOCCI.

It has already been stated that when ears, which had been locally protected by recent erysipelas, were again inoculated with living streptococci, a more or less severe inflammatory reaction followed, and that this appeared before any inflammation was observed in the opposite ears of the same animals, treated in the same way, or in those of the controls. And we have given as a reason for thinking that this inflammation was not mild erysipelas, the fact that no multiplication and distribution of the organisms in the lymphatic spaces of the ear could be observed. It remained to be seen whether the poisonous products which had been introduced with the living cocci were capable of causing this reaction. We accordingly inoculated, with concentrated filtered cultures, both ears of rabbits which had recently recovered from erysipelas in one ear (right). The details of the four experiments which we made are given in Table V.; and the changes in volume which resulted in two of them are illustrated by curves (Figs. 6 and 7).

TABLE V.—Comparing the Effects produced by inoculating Filtered Cultures into both Ears of Rabbits which had recovered from Erysipelas in the Right Ear.

Animal.	Result of First Inoculation with Streptococci.	Interval between Inoculation.	Dose in Minims.	Inoculation of both Ears with Broth from filtered Cultures, concentrated <i>in vacuo</i> to $\frac{1}{15}$ of its original volume.	
	Right Ear only.			Right Ear.	Left Ear.
A.	Erysipelas (severe).	18 days.	15	Severe inflammatory reaction, subsiding third day.	Less severe inflammatory reaction than in the right, slower in its development, but subsiding same day.
B.	Erysipelas (severe).	13 days.	4	Severe inflammatory reaction, subsiding second day.	Inflammatory reaction, never quite so severe as in the right, but not subsiding till fourth day.
C.	Erysipelas (severe).	16 days.	15	Severe inflammatory reaction, subsiding second day.	Very slight reaction.
D.	Erysipelas (severe).	26 days.	3	Severe inflammatory reaction, subsiding third day.	Inflammatory reaction much less severe than in the right, subsiding third day.

The following curves contrast the changes in volume which occurred in the two ears of a rabbit, which had recently recovered from erysipelas in one of them (right), as the result of injecting filtered cultures of streptococci, concentrated to $\frac{1}{15}$ of their original volume:—

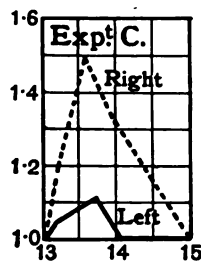


FIG. 6.—Dose = 15 drops.

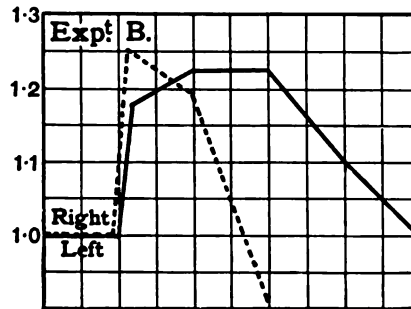


FIG. 7.—Dose = 4 drops.

In these experiments a marked but transient inflammatory reaction occurred in the ears previously affected by erysipelas, while a less severe, but in some cases a more prolonged, reaction occurred in the others; and they clearly showed that the ears locally protected against erysipelas react more strongly than their fellows, which are less

effectually immunised, to the irritation caused by the chemical products of the streptococcus.

In the majority of our experiments, however, inoculations had been made with the cocci from agar cultures, and presumably therefore little or no extra-cellular products of microbic activity had been introduced. Klein¹ has demonstrated the existence of intracellular poisons in certain microbes, and this has been confirmed by Kanthack and Wesbrook.² And it occurred to us to inquire whether an injection of dead streptococci, in quantities as small as those used in the experiments alluded to, was capable of causing inflammation, and if so whether we should observe the same differences in the early reaction of the two ears which we observed when living microbes were used. We accordingly inoculated the two ears of a rabbit which had recently recovered from erysipelas in one ear (right) with minute quantities of streptococci from an agar culture sterilised in the following way. The cocci were suspended in a minute quantity of sterilised water, and drawn up into a capillary pipette. This having been sealed, was put into a water bath at a temperature of 55° C., and allowed to remain there 10 minutes. That the cocci had been destroyed by this procedure was proved by sowing them on agar tubes. No growth appeared in these, while a control tube, sown with the material before heating, developed a good growth. A single drop of the fluid containing the dead cocci was then injected into each ear.

TABLE VI.—*Result of Inoculating with Streptococci killed by Heat both Ears of a Rabbit which had recently recovered from Erysipelas in the Right Ear.*

Interval since Erysipelas in Right ear.	Dose.	Result in Right Ear.	Result in Left Ear.
18 days.	1 drop.	Severe inflammatory reaction, ending third day.	Milder inflammatory reaction, commencing slowly but lasting till fifth day.

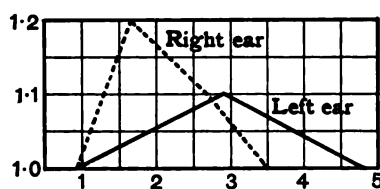


FIG. 8.—Curves of volume from the same case (in which dead streptococci were used).

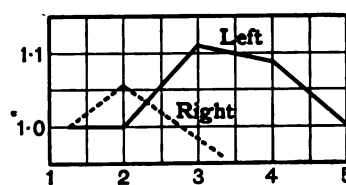


FIG. 9.—Curves of volume obtained when living streptococci were used. (From one of the cases in Table III. Class 2.)

In this ear the result produced was exactly the same as if a similar quantity of living cocci had been injected. The usual inflammatory

¹ *Brit. Med. Journ.* March 25th, 1893.

² Kanthack and Wesbrook, *Brit. Med. Journ.* Sept. 9th, 1893.

reaction appeared after a few hours, reached its maximum on the second day, was fading on the third, and had disappeared on the fourth. In the left, a little patch of redness about the seat of inoculation was seen on the second day, had slightly increased on the third, and did not disappear till the fifth. This experiment showed that the inflammatory reaction which we observe, when immunised ears are injected with living streptococci, may be caused by the poisons contained in the microbes actually introduced. This, together with the fact that we could obtain no growth of micro-organisms when we sowed the oedema fluid of such ears on nutrient media, finally convinced us that no multiplication of the microbes takes place when they are introduced into immunised ears.

XII. ON THE PRODUCTION OF GENERAL IMMUNITY BY INTRA-PERITONEAL INJECTIONS OF ATTENUATED CULTURES OF STREPTOCOCCUS ERYSIPELATIS.

We may now proceed to inquire whether rabbits which had recovered from intra-peritoneal injections had acquired any general immunity. In order to investigate this point we inoculated their ears with virulent cultures, control animals being in all cases similarly treated.

In the experiments given in Table VII. the results were similar to those obtained when a rabbit which had recovered from erysipelas in the right ear was inoculated in the left, before the period of general immunity had elapsed; that is to say, a more or less severe inflammatory reaction appeared before any inflammation was observed in the controls, and subsided on the second or third day. In two only of the nine cases did erysipelas result, and in one of these, the long interval of 80 days having elapsed between the inoculations, the period of immunity was probably at an end. On the other hand, erysipelas appeared in all the control animals, and ended fatally in six.

In these cases the general immunity was similar to but more complete than that conferred by an attack of cutaneous erysipelas. It was more complete, possibly because the area of peritoneum affected was very much larger than that of a single ear; but we must also point out that the majority of the animals had had several intra-peritoneal injections before their general immunity was tested, while in the other series of cases the ears shared only in whatever general immunity had been caused by one attack of erysipelas in the opposite ears.

[TABLE.]

TABLE VII.—*Showing the General Immunity conferred upon Rabbits by Intra-Peritoneal Injections of Cultures of Streptococcus erysipelatis.*

Animal.	Number of previous Intra-Peritoneal Injections.	Interval between Inoculation of Ear and the last Intra-Peritoneal Injection.	Result of Inoculation of Right Ear.	Control Animal.
I.	2	7 days.	Inflammatory reaction (lasting 3 days).	Erysipelas, severe (subsiding ninth day).
II.	2	6 days.	Inflammatory reaction (3 days).	Erysipelas, severe (subsiding ninth day).
III.	2	2 days.	Inflammatory reaction (3 days).	Erysipelas (death eighth day).
IV.	2	6 days.	Inflammatory reaction (4 days).	Erysipelas, mild (subsiding eighth day).
V.	2	10 days.	Inflammatory reaction (2 days).	Erysipelas (death eighth day).
VI.	4	3 days.	Inflammatory reaction (4 days).	Erysipelas (death, eleventh day).
VII.	4	3 days.	Inflammatory reaction (2 days).	Erysipelas (death, fifth day).
VIII.	1	4 days.	Erysipelas (subsiding seventh day).	2 Controls { 1. Erysipelas (death, fourth day). 2. Erysipelas (subsiding eighth day).
IX.	1	80 days.	Erysipelas which spread to opposite ear (death eighth day, cocci in blood).	Erysipelas (death, eighth day).

XIII. ON THE RELATION OF THE EARLY INFLAMMATORY REACTION TO IMMUNITY.

The theory that inflammation is a protective process has commended itself to many observers, and it seems worth while to inquire whether our experiments give any support to this view. We have already pointed out that when the ears of immunised and of normal rabbits were inoculated, the appearances of inflammation were first seen in the former. This difference was very striking when ears locally protected were compared with those of the control animals. The inflammatory reaction varied considerably in intensity in different cases, but was constantly present, and in most instances was subsiding at a time when inflammation had made but little progress in the controls. It will be remembered that these cases proved to be absolutely immune. Again,

when ears which shared in the general immunity which followed one or more intra-abdominal injections were inoculated and compared with those of control animals, a similar early inflammatory reaction appeared in the former before inflammation had made much progress in the latter. The difference in these cases was not quite so striking, and was altogether absent in the case of the animals (Table IV. Nos. 8 and 9) which developed erysipelas, and also in the case of No. 4, which proved immune, and whose inflammatory reaction was delayed until the third day.

On the other hand, this early inflammatory reaction was not nearly so striking in the case of ears which shared in whatever general immunity followed cutaneous erysipelas in the opposite ear, and we may repeat that these ears proved less immune than those alluded to above.

We may state then, briefly, that the rapidity of the onset of inflammation which followed the inoculation with streptococci was in proportion to the immunity observed, and we think it probable that the early inflammation, by drawing to the spot a large number of leucocytes, and of possibly bactericidal serum,¹ was the means by which the part resisted the micro-organisms before they had sufficient time to multiply and generate their poisons; in other words, that a rapid inflammatory reaction is the chief factor in the production of the immunity, both local and general, which we observed in our experiments.

XIV. ON THE RELATION OF INFLAMMATION TO RECOVERY FROM ERYSIPELAS.

It has long been known that in erysipelas the streptococci may be found near the margin of the inflamed area with greater certainty than in parts which have been longer affected. We have made some experiments in order to determine how long they remain alive at any one spot, and have found that œdema fluid, taken from a part which, on the previous day, corresponded to the visible margin, almost invariably contained living cocci, while fluid taken from the same spot 24 hours later—that is to say, when redness has replaced the paleness for about 12 hours—usually contained none. In one instance, however, they were found in a part where redness had existed for 24 hours, but they had disappeared by the following day. These observations furnish additional evidence that recovery of the parts first attacked commences while the disease is spreading elsewhere.

We would ask, What is the cause of this local recovery, and also of the ultimate cessation of the spread of erysipelas? In the earlier part of this paper we suggested that the cause of recovery and subsequent immunity are probably identical, and we have given reasons for thinking that immunity is brought about by an inflammatory reaction which destroys the micro-organisms and arrests the disease at its outset.

¹ Roger has shown that *Streptococcus erysipelatis* grows well on the serum of immune rabbits, but becomes attenuated in virulence.

It now remains to be seen whether there is any evidence that recovery is also due to inflammation. Let us first consider the case of recovery which occurs in the parts first affected by erysipelas while the disease is still spreading elsewhere. This we shall speak of for the sake of brevity as "local recovery." If we watch the changes which occur in a small portion of a rabbit's ear, from the very commencement of erysipelas in it, we notice that the part first becomes cedematous, but remains pale, and that it contains living streptococci in large numbers. After about 12 hours, the pallor is replaced by redness, and when this redness has persisted for about 24 hours, living micro-organisms can no longer be found there. It seems reasonable to suppose, therefore, that the inflammation has brought about their destruction; that it is, in fact, the cause of local recovery.

The cause of the ultimate cessation of the disease appears to us to be similar.

We have seen that when erysipelas is actively spreading, the affected area is bounded by a pale cedematous zone, in which the micro-organisms can readily be found; in other words, that the advance of the virus precedes the advance of the redness, and we have also seen that when a part efficiently protected by general immunity, produced by erysipelas elsewhere, is again inoculated, an inflammatory reaction occurs which is characterised by the absence of the pale zone containing cocci, and that the inflammation occurs sooner than in the control animals inoculated for the first time. Is it not, therefore, reasonable to suppose that this power of reacting quickly to the irritation of the streptococci is acquired during the course of the disease, and is the means of arresting it. We suppose that, as the cocci spread in the lymphatic spaces of the tissues, they are followed by inflammation more and more closely, until reaction at last becomes coincident with invasion, and the disease is brought to an end, for on more than one occasion we have observed that, towards the end of an attack, the inflamed area was no longer bordered by a pale zone of cedema, but redness extended to the very margin of the affected part. We did not observe this more frequently, because erysipelas starting in the ear of a rabbit does not cease to advance until it has spread to the head and neck, where the character of the skin renders the margin less distinct, and where it can only be seen at all after the hair has been removed.

It was not without considerable hesitation that we arrived at the conclusion that inflammation was the cause both of recovery and of immunity. We were aware that an actively inflamed part has long been regarded as a place of lessened resistance and particularly susceptible to invasion by micro-organisms, and we ourselves have seen a rabbit's ear, while inflamed by previous immersion in warm water, become attacked by erysipelas. We have pointed out that in our experiments inflammation produced by introducing streptococci into a locally immunised ear is followed by rapid destruction of the microbes, while, on the other

hand, we find that streptococci can attack an ear already actively inflamed and produce erysipelas, and it was necessary to inquire into the cause of this apparent contradiction before we could maintain with any confidence the theory that inflammation is protective.

Modern pathology teaches that a transient injury, however severe, is unable of itself to produce inflammation; that to cause inflammation the irritant must act for a considerable time. The view that inflammation is a protective reaction on the part of the tissues, the purpose of which is to get rid of the source of irritation, is becoming more and more widely accepted.¹ A wound caused by a transient injury does not inflame if it remains aseptic; and whatever inflammation follows a bruise is to be attributed, not directly to the injury, but to the presence of extravasated blood and other dead matter in the injured tissue.

Let us examine, in the light of this theory of inflammation, what takes place in a rabbit's ear which has been injured by immersion in warm water. It at first becomes intensely hyperæmic, but this passes off in a few minutes. It is then found to be studded with petechial hæmorrhages, and very soon becomes swollen with œdema. This hæmorrhage and œdema we may regard as the direct result of damage done to the tissues, and not as a part of the inflammatory process which follows later, and whose purpose it is to remove these effete matters. If micro-organisms be introduced during the early part of the inflammatory stage, they find in the œdema fluid a favourable medium for their growth, and the inflammation which already exists, probably only sufficient to effect the purpose for which it was called forth, has now to cope, not only with the extravasated matters, but also with the cocci. Further inflammation is accordingly excited, but not more rapidly than in uninjured tissues to which streptococci have found their way; and so, as in this latter case, the micro-organisms gain a start and erysipelas is the result. It is quite otherwise when an ear immunised by previous erysipelas is again inoculated with streptococci: then, as we have shown, the inflammation starts far more quickly, and is able to destroy the microbes before they have had time to multiply and generate their poisons.

Bearing these considerations in mind, we came to the conclusion that the fact that streptococci may attack a part already inflamed is not really opposed to the theory that inflammation is protective.

XV. ON THE IMMUNITY PRODUCED BY CHEMICAL PRODUCTS OF THE STREPTOCOCCUS ERYSIPELATIS.

The injection of a filtered beef-broth culture into the peritoneal cavity, was found to render an animal immune to a subsequent injection

¹ Thus Metchnikoff defines inflammation as a "phagocytic reaction on the part of the organism against irritants," "Comparative Pathology of Inflammation," 1891, Eng. Trans. p. 189.

of a living culture in the same situation, a similar dose of which proved fatal to a control animal (Table II. p. 50).

Roger¹ has pointed out that an injection of a filtered culture of streptococci into the veins, renders a rabbit more susceptible than before to the action of the living virus, and that this increased susceptibility persists for at least two months. The experiment which we have mentioned above will not appear incompatible with his results, if we bear in mind that the probable effect of the intra-abdominal injection of the filtered culture was an inflammation of the peritoneum, which gave rise to a local immunity, and we must point out that we made no attempt to determine whether the injection had conferred any protection upon other parts.

The same material, when injected into the ear, caused inflammation of greater or less intensity, according to the dose employed, and this was confined to the neighbourhood of the seat of injection, and rapidly subsided. We hoped, by this means, to be able to cause a local immunity in the tissues in advance of the spreading erysipelas, and so check its further course. Five attempts have already been made, but up to the present time we have not met with much success. In all instances we inoculated the ear with sterilised products near the root, and subsequently inoculated the tip with living virus. The erysipelas which developed appeared to spread over the whole ear, but we did not determine whether the part directly affected by the first inoculation had been invaded by micro-organisms, nor could we tell whether this inoculation with sterilised products had checked the advance of erysipelas, because in rabbits the disease seldom spreads beyond the ear. It is worthy of note, however, that none of these animals died, although locally the disease was severe.

CONCLUSIONS.

1. That cultures of *Streptococcus erysipelatis* cease to grow in beef-broth after 3 or 4 days, and that the majority of the cocci die a few days later; but a few remain alive and capable of producing a new growth when sown on new culture media, for 90 days, and probably longer.
2. That the cause of cessation of growth in beef-broth is an exhaustion of the nutrient material, and not the production of a bactericidal substance.
3. That cultures of *Streptococcus erysipelatis* maintain their virulence when grown on solid media, but become greatly attenuated when grown in beef-broth.
4. That the virus which has become attenuated by cultivation in beef-broth may be intensified by growing in the bodies of animals.
5. That the injection of streptococci or their products into the

¹ Roger, *loc. cit.*

abdominal cavity confers immunity to a second injection, in the same situation, of more virulent cultures, in quantities fatal to control animals.

6. That cutaneous erysipelas completely protects the parts directly affected against subsequent inoculations of the virus. In other words, it confers an absolute local immunity, while on the rest of the body it confers a general immunity which is less constant, sometimes protecting completely, at other times modifying, the course of the disease, while in some instances it is entirely absent.

7. That intra-abdominal injections of attenuated cultures of streptococci confer a somewhat more perfect immunity upon the rest of the body.

8. Both local and general immunity are of short duration, and do not last more than a few weeks.

9. That when streptococci are introduced into rabbit's ears, protected by recent erysipelas, an inflammatory reaction quickly appears, and has already subsided before inflammation has made any considerable progress in the control.

10. That the rapidity of onset, and the intensity of this inflammatory reaction, is most marked in ears locally protected, less marked in those which share in the general immunity produced by intra-peritoneal injections, and least marked in those which share in the general immunity produced by erysipelas in the opposite ear; that is to say, it is in proportion to the immunity observed in these three classes of cases.

11. A similar difference in the inflammatory reaction of immunised and normal parts is observed when filtered cultures or dead streptococci are introduced.

12. That this power of quickly reacting is an important factor of immunity both general and local.

13. That this same power of reacting is acquired during the course of the disease, and is the cause of recovery.

SOME OF THE EFFECTS OF SUNLIGHT ON TETANUS CULTURES.

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SUNLIGHT as a germicidal agent has long furnished a subject of great interest, not only to the bacteriologist, but to everyone who is interested in hygienic and disinfecting methods. The work of recent and earlier investigators has shown with what an exceedingly strong and efficacious method nature has provided us, and in this instance given us support, gained by scientific experiment, for the popular idea that sunlight and fresh air are the enemies of disease.

The researches of Downes and Blunt¹ in 1877 aroused an interest which has never since ceased to exist. Roux² confirmed their results by showing, in the course of some very interesting experiments, the necessity not only of the light, but of air (or oxygen) in this destructive process. He showed that anthrax spores were completely destroyed when exposed to light and air, but were unaffected when air was excluded. Many other observers, notably Arloing,³ Janowski,⁴ Buchner,⁵ Marshall Ward,⁶ and others have contributed to our knowledge of the effects of sunlight on micro-organisms, though their observations were nearly all made on aërobic bacteria.

Kitasato,⁷ Tizzoni and Cattani,⁸ Pernossi and Fermi⁹ have made

¹ "Researches on the Effects of Light upon Bacteria and other Organisms," *Proc. Roy. Soc. London*, 6th Dec. 1877, vol. xxvi. No. 184.

² Roux, "De l'Action de la lumière et de l'air sur les spores de la Bactérie du Charbon," *Ann. de l'Inst. Pasteur*, Paris, 1887, pp. 445-452.

³ Arloing, *Arch. de physiol. norm. et path.*, Paris, 1886, p. 209.

⁴ Janowski, "Zur Biologie der Typhusbacillen," *Centralbl. f. Bakteriologie u. Parasitenk.* 1890, bd. viii. Nos. 6-9.

⁵ Buchner, "Ueber den Einfluss des Lichtes auf Bakterien," *Centralbl. f. Bakteriologie u. Parasitenk.*, Jena, bd. xi. No. 25, and bd. xii. Nos. 7 and 8.

⁶ Marshall Ward, "Experiments on the Action of Light on *Bacillus anthracis*" (Communication made to the Royal Society, Dec. 1892).

⁷ Kitasato, "Experimentelle Untersuchungen ueber das Tetanusgift," *Ztschr. f. Hyg.*, Leipzig, 1891, bd. x. ss. 287-289.

⁸ Tizzoni and Cattani, *Arch. f. exper. Path. u. Pharmacol.*, Leipzig, bd. xxviii. s. 41.

⁹ Pernossi and Fermi, "Ueber das Tetanusgift," *Ztschr. f. Hyg.*, Leipzig, 1894, bd. xvi. ss. 393-396.

experiments, however, with tetanus cultures, and have shown that light and air when acting together will destroy the poison in a very short time. In many cases the researches have been principally limited to the determination of the actual effects of light on the life of the micro-organism, as indicated by its delay in development or complete destruction, and the effects on the pathogenic properties have been in most instances somewhat overlooked.

In the tetanus bacillus we have a micro-organism which produces such characteristic symptoms, and is fatal in such minute doses, that it is particularly well suited for research respecting any changes in virulence which might be brought about by the action of sunlight, and on this account I have employed it in nearly all my experiments.

The primary object of the present investigation was to repeat (in a somewhat modified form) for tetanus what Roux had done with anthrax, and as might be expected it was found that old broth cultures, on exposure to the sunlight in an atmosphere of hydrogen, were not in the least affected either in regard to their virulence or their rapidity of growth on reinoculation. When, however, the same culture was sealed up in the presence of air, the micro-organisms were not only killed, but the material was rendered completely harmless when inoculated into white mice.

The complete destruction of the micro-organism and the disappearance of virulence were not, however, synchronous, as it was found possible to obtain vigorous and virulent growths from cultures which had been rendered quite innocuous by the action of the sun.

Although the poison is so readily killed by the action of light, one has always associated with tetanus the idea that it was so malignant and virulent in its action, that the lethal dose must be incalculably small, though Vaillard and Vincent¹ have endeavoured to show that the spores, deprived of the toxin, are not harmful to animals.

The results of these present experiments might perhaps seem in some measure to support this conclusion, for on administering subcutaneously to mice a culture of tetanus, which had been exposed to the action of sunlight in the presence of air for some days, they exhibited absolutely no symptoms, while the same material which had been exposed to light in hydrogen, and that also exposed to air in the absence of light for the same time, produced tetanic symptoms and fatal results when given in the same doses. That there were many living spores in the .3 c.c. of innocuous material, which each animal received, was demonstrated by obtaining vigorous growths of typical tetanus bacilli when fresh cultures were made. The fresh tubes were sown either with one platinum wire-loop, or by using simply the material which adhered to a straight platinum needle, which was dipped once into the culture.

That the actual virulence of the spores may have been decreased by

¹ Vaillard and Vincent, "Contribution à l'étude du Tetanus, 1re Memoire," *Ann. de l'Inst. Pasteur*, Paris, Jan. 1891.

the sunlight, and their power of multiplying in the animal tissue thus destroyed, is quite possible, though it was observed that the fresh growths were not only quite as virulent as before, but were even more potent and killed in a shorter time.

On beginning the experiments, .3 c.c. of a broth culture, which was three months and a half old, killed mice in 20–30 hours, and the final growth now in the same doses and in cultures of 2 weeks old will kill in 12 hours.

On reinoculation into fresh bouillon, there was a quite perceptible delay in the development of those bacteria which had been for a long time exposed to sun and air, which would seem to accord with the results obtained by Janowski¹ when working with typhoid cultures.

On observing how essential was the presence of oxygen to this destruction of tetanus poison and spores by light, there seemed a possibility that oxygen might actually be used up during the process, and an apparatus was made by which a diminution in the volume of the contained air could be demonstrated by means of a manometer.

The rate of the destructive process was found to be dependent on many variable factors. The concentration of the culture, the relative quantities of air and fluid, together with the surface area relative to the amount of fluid contained, had each an influence on the rapidity of destruction.

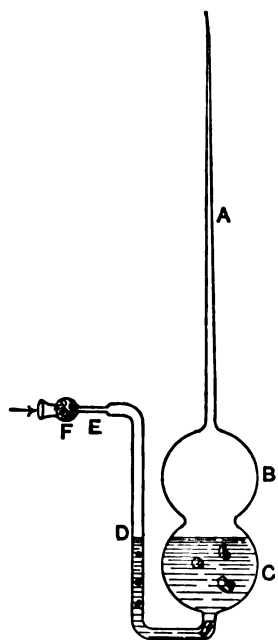


FIG. 1.

METHODS EMPLOYED.

Cultures were always grown in the ordinary dextrose bouillon, either in Liborius tubes, or in a simple manner as follows:—

An ordinary pipette, such as one uses constantly for transferring in a sterile manner the contents of one tube to another, was bent, as in Fig. 1.

The dextrose broth is inoculated with tetanus in an ordinary test tube, and then by inverting the pipette the fine tube A can be placed in the fluid, and it is thus easily drawn up in the bulbs B and C by sucking at F.

It is now turned as in the figure, and hydrogen is bubbled through F into the culture medium and out through tube A, which should be previously sterilised and dried in the flame to prevent small drops of the inoculated material being scattered over the operator and furniture.

¹ Janowski, "Zur Biologie der Typhusbacillen," *Centralbl. f. Bakteriol. u. Parasitenk.* Jena, 1890, bd. viii. Nos. 6, 7, 8, and 9.

The tubes are then fused at A and E.

It is really but a modification of Roux's¹ method, but has the advantage of being simple, and anyone who can use a blowpipe can make it for himself.

It can be made of any size, and is sterile and ready for use when completed.

The cultures which were exposed to the light were always sealed up either in air or in an atmosphere of hydrogen. Mice were the animals employed for experiment, and the inoculations were always made subcutaneously in the back.

EFFECT OF LIGHT ON PATHOGENESIS AND GROWTH.

A culture of the tetanus bacillus which had been grown for 3 months in dextrose bouillon was divided into two equal parts, and placed in pipettes, as previously described and figured. In one was air, and in the other hydrogen. The pipettes were fused up at both ends, and exposed freely to the action of the sun, so hanging that they were on the side (so as to give greatest possible surface), and kept constantly agitated by the wind. At the end of 19 days they were opened and fresh cultures made, and 2 mice inoculated with each.

The mice each received .3 c.c. subcutaneously in the back. The culture which had been exposed to the light in hydrogen produced tetanus and death in 1 mouse in 22½ hours, and the other was killed by decapitation at the end of 25 hours, when it was just dying.

The 2 mice which had received the tetanus exposed to light in air never showed any symptoms at any time, though under observation for 6 weeks.

From both these samples, however, cultures were obtained of perfectly typical tetanus, and the culture from the material which was previously innocuous produced, on inoculation in same manner and dose into 2 mice, tetanus and death in 16 and 20 hours respectively.

At the end of two months and a half, one of the cultures which was made from the harmless material was opened, and 5 c.c. placed in an ordinary test tube, which was then fused up in a flame, so that the tube contained 5 c.c. of liquid and 10 c.c. air, and this was suspended in the sun so as to be gently agitated by the wind.

In 3 days the tube was opened, and a mouse inoculated with .3 c.c. of the contents, which sufficed to kill it in 25½ hours. Another mouse received the same dose of the same culture which had been exposed to air in the dark, and succumbed in 19 hours.

The material was again sealed up, and placed in the light for 9 days longer, when 2 mice were inoculated, each with .3 c.c. They exhibited no symptoms at any time.

¹ Roux, "Sur la Culture des Microbes Anaérobies," *Ann. de l'Inst. Pasteur*, Paris, Feb. 1887, p. 59.

Although this material had been quite harmless to the mice, good cultures of typical tetanus were obtained from it. The fresh tubes were sown with that amount only of the material which adhered to a straight platinum needle.

These fresh cultures after growing for 2 weeks, when inoculated subcutaneously, in doses of .3 c.c. into 3 mice, killed all in 12 hours. Two of the 3 mice thus tested and killed were those 2 which had 2 weeks previously survived the administration of the harmless material, and it was shown that they at least were not immune to tetanus.

Why were they not killed on receiving the first dose of tetanus spores? The spores were living and capable of developing in fresh bouillon, if not in the animal body. Were they immune to spores deprived of poison, or was the dose a sublethal one, or were the spores temporarily attenuated?

If the spores were attenuated, the cultures obtained from them were at least not attenuated, but hyper-virulent.

The animals must have received many of these living spores, when that amount of the material which adhered to a straight platinum needle, sufficed for reinoculating fresh tubes. If the result obtained was due to administration of a sublethal dose it would be of interest to know just what the minimum lethal dose for mice is.

The other possible explanation is that tetanus spores without poison are harmless when injected into mice.

These experiments have not at present been carried far enough to enable one to give a definite answer to the question, but further research will perhaps afford the explanation.

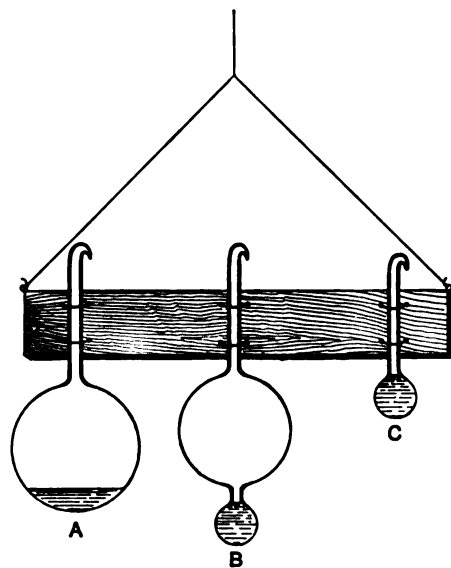


FIG 2.

RELATION OF SURFACE AREA TO RAPIDITY OF DESTRUCTION.

In order to observe the effect of varying the surface area in the same amount of contained culture, the following experiment was done.

Three small glass vessels were blown, which are represented in the Fig. 2, A, B, and C. The total capacity of A was 21.7 c.c.; capacity of the larger bulb in B was 10.6, and of the smaller 1.6 c.c.; capacity of C was 1.6 c.c. The neck of C corresponded in diameter (about $\frac{1}{8}$ in.) with the constriction joining the

large and small bulbs in B. All these vessels were plugged with cotton-wool, and sterilised after the capacity had been determined.

By means of a sterile capillary pipette, capable of containing 1·6 c.c., the same quantity 1·6 c.c. of the same culture was placed in A, B, and C. All were then sealed up in a flame.

In A there was 1·6 c.c. of an old tetanus culture and 20·1 c.c. of air, so arranged that the surface area was very great. In B was 1·6 c.c. culture and 10·6 c.c. air, and here the surface area exposed to the air corresponded to that in C. In C was 1·6 c.c. culture and ·1 c.c. air.

These bulbs were fixed in the manner shown in the figure to a piece of wood, and the whole suspended freely by a string so as to allow of a rotatory movement when acted on by the wind.

On exposure to the sun for 18 days there was complete destruction of the micro-organism in A, so that no growth could be obtained, while from B and C good cultures were easily obtained.

A METHOD OF SHOWING A DIMINUTION IN THE VOLUME OF THE AIR IN WHICH THE TETANUS CULTURE IS EXPOSED TO THE SUN.

In Fig. 3 is represented a simple apparatus by which it is possible to show that during the exposure of a sealed vessel, containing air and culture, to the sunlight, an actual diminution in the volume of the air takes place.

The apparatus consist of 2 bulbs, joined by a tube which for part of its length is composed of capillary or thermometer tubing, bent into the shape of a U.

From the bulb B, which is to contain the culture and air, near its junction with the tube H, is given off a smaller tube F, through which the tetanus culture can be drawn up into B. This small tube is fused at its lower end, and is bent, as represented in the figure, for the purpose of convenience in manipulation. Opening from the bulb A, which contains air alone, is a tube E, which is constricted at G, and has a cotton-wool plug at D.

The whole apparatus when made can be sterilised in a hot-air steriliser, and is then ready for use. It can be supported in an ordinary wooden stand with clamp, such as is used for burettes, and should be always kept in the same

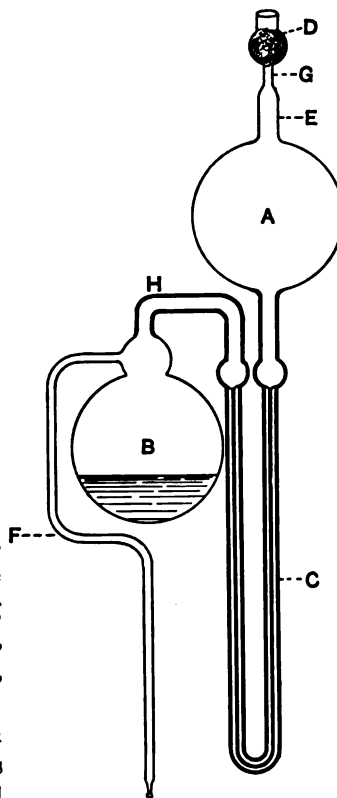


FIG. 3.

stand, and the readings taken on the same table so as to avoid errors in position (deflection from the perpendicular).

To use it, the fused point of F is broken off, and some of the culture upon which it is proposed to make the observation is drawn up into the bulb B, by exhausting the air in A through the tube D, which is placed in the mouth. When sufficient culture has been drawn up into B, the fluid which remains in F is expelled by blowing, and then F is sterilised in the flame.

The cotton-wool is removed from the tube D, and a small quantity of mercury is placed in the manometer C, by means of a capillary pipette, and the tube D is drawn out at the constriction G to a very fine point, which is, however, not sealed.

The whole apparatus is now allowed to stand in a place free from draughts until it is cooled or heated throughout to the same temperature. It is now sealed quickly in a very small blowpipe flame at G and at the lower end of F.

After it has stood for some hours in a dark place, in which the temperature is constant, the height of the mercury is marked in each limb of the manometer, and it will be found on keeping this in the dark that the height will not vary even on placing the apparatus in the incubator. There will, at first, be a temporary alteration, due to the air in A being heated more quickly than the fluid in B, but after a few hours, either in the incubator or in the cold, the mercury always returns to the same level, so long as it is not exposed to the sunlight.

On exposing the culture, thus sealed up, to the sunlight, within a very short time there is an alteration in the level of the mercury, which begins gradually to rise towards the bulb containing the culture. To take the reading after exposure to sunlight, the apparatus must again be allowed to become evenly cooled or heated throughout. By this means it is possible to get as great a difference as 2 in. between the preliminary and final reading, after prolonged exposure to the sun's action.

If through carelessness in manipulation the culture becomes contaminated, the readings will be inaccurate, as it is impossible to get a correct preliminary reading when some of the gas-forming bacteria begin to grow in the fluid, and cause the mercury to rise towards A.

As a control, distilled water was placed in a similar apparatus, and no rise in either limb was observed even after several weeks.

With sterile broth almost the same result was obtained as with culture, so that the following experiment was carried out as a control.

The apparatus was so modified, that the 2 bulbs were *quite the same* as B, and of equal size; in the position occupied by A in the figure was simply a tube, constricted, and containing cotton-wool at its upper extremity.

In A was placed 10 c.c. of an old culture of cholera in Uschinsky's fluid, in which growth had long since ceased, and in the other bulb B, 10 c.c. of sterile Uschinsky's fluid. The mercury was put into the

manometer, and after sealing up and exposing to the sunlight, it was observed that the mercury rose in the limb towards the culture, and away from the sterile fluid, thus indicating that the diminution in volume was more rapid in that bulb which contained the culture than in the one which contained culture medium alone.

It may be mentioned here, that the anærobic cultures, after being taken from the atmosphere of hydrogen, were allowed to stand for some days in ordinary test tubes stoppered with cotton-wool. This was in order that there might be no possible error introduced by the absorption of oxygen after sealing up the apparatus.

The cholera culture was used, because at the time no old broth culture of tetanus was available.

CONCLUSIONS.

The results obtained in these experiments confirm very strongly the conclusion arrived at by Downes and Blunt,¹ Duclaux,² Roux,³ and others, that oxygen is a necessary factor in the destruction of bacteria by light. Not only is this so, but an actual diminution of the contained air was shown to take place during the time of exposure of cultures in air.

Whatever the process consists in, the larger the surface exposed the more rapid is the destruction when equal volumes are exposed.

At present we have not sufficient data to say exactly what this process is any more than to enable us to know that in the absence of oxygen the sun is powerless to produce any harmful effect on the bacteria exposed to it.

Tetanus cultures are completely killed on prolonged exposure to the sun in air, though it is possible to stop at a point where the pathogenesis vanishes, while life still remains in the spores.

From the limited number of observations it would appear as if those spores which survive the sun's action are capable of producing, on re-inoculation into fresh tubes, not only quite as virulent cultures as before, but the pathogenesis seems to be increased.

It is also very evident that it is possible to inject into mice living tetanus spores without producing any symptoms of any kind.

Whether this is due to the smallness of the dose, to a temporary attenuation, or to the fact that in the absence of the poison tetanus spores are harmless, is a question which at present must remain unanswered, but must be left until further research throws more light upon it.

¹ Downes and Blunt, *loc. cit.*

² Duclaux, *Compt. rend. Acad. d. sc.*, Paris, tome c. and ci.

³ Roux, *loc. cit.*

A REPORT OF TWO CASES OF ACTINOMYCOSIS OF THE BRAIN.

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From the Pathological Anatomical Institute of Professor Chiari in Prague.

FOR the material from these cases, as well as for much kind advice and assistance, I am indebted to Professor Chiari, in whose institute the cases were first examined and the autopsies made.

Merely a cursory glance over the total number of cases of actinomycosis in man, hitherto published, is necessary to convince one how comparatively rare are metastases in any form, the affected organs in most of such instances belonging to the thoracic and abdominal cavities. In the brain, however, metastases are peculiarly rare, and the most careful perusal of the various monographs and compilations on the disease fails to reveal more than 3, or at most 4, cases of the kind.

Ponfick,¹ in his well-known work, describes the oft-quoted case (Frau Deutschmann), in which, among other metastases, there were found, in the brain, abscesses containing the actinomyces fungus. Apart from this, however, the author had observed no case of a similar nature, though he further records an instance in which the disease, having commenced in the prevertebral region, advanced *per continuitatem* to the brain and meninges (August Barunké).

J. Israel,² who in 1885 had collected 38 cases, mentions but one other instance of actinomycotic metastases in the brain, observed and placed on record by König and O. Israel.³ Here the disease, as viewed by J. Israel, having commenced in the lungs, was propagated through blood channels to various organs of the body, involving likewise the brain and its membranes.

In this work is further cited the case observed by Zemann,⁴ and considered by him as primary actinomycosis of the Fallopian tube, with the formation of secondary abscesses in the liver, lungs, and brain.

¹ Ponfick, "Die Aktinomykose des Menschen; eine neue Infektions-krankheit." Berlin, 1882.

² J. Israel, "Klinische beiträge zur Kenntniss der Actinomykose des Menschen."

³ König and O. Israel—König, "Ein fall von Actinomykosis hominis." Inaug. Diss. Berlin, 1884. O. Israel, *Berl. klin. Wchnschr.* 1884, No. 23.

⁴ Zemann, "Ueber die Aktinomykose des Bauchfells und der Baueingeweide beim Menschen," *Wien. med. Jahrb.* 1883.

That these abscesses, however, are to be regarded as actinomycotic is uncertain, in that the author failed to discover in any of these metastases the parasite which excited the primary lesions.

J. Israel himself had never observed any case of actinomycosis with cerebral abscesses, nor has Boström,¹ in his otherwise exhaustive and elaborate treatise on the subject, made any mention of their occurrence.

There remains, lastly, to be mentioned the interesting article by Bollinger,² describing a primary actinomycosis of the brain, in which the only discernible lesion was an actinomycoma, situated between the anterior pillars of the fornix.

Considering, then, the marked rarity of cases such as those here mentioned, and that they, moreover, present certain peculiarities which differentiate them from others, the subjoined communication may perhaps be justifiable.

CASE 1.³—W. W., æt. 38, a blacksmith; had been for some time under treatment in the klinik of Prof. Gussenbauer, on account of a phlegmonous condition of the sternum and tissues about it—of 5 months' duration. This was incised and treated in the usual manner. The patient, however, became gradually worse, presented symptoms pointing to pulmonary tuberculosis, and finally, 3 weeks after the operation, died, with evident signs of a complicating tubercular affection of the meninges.

The autopsy (performed as a class exercise, November 15th, 1886) presented the following conditions:—

The *body* was that of a medium-sized well-built man; emaciated; post-mortem lividity in dependent parts. Pupils somewhat contracted and equal. Thorax well developed; on the anterior surface in the region of the manubrium sterni and corpus was a large ulcerated area, 10 cm. in diameter, involving the skin and subcutaneous tissue, and extending in several places to the subjacent bone and ribs. A sinus from here communicated with the thoracic cavity. From the right border of the ulcer was an incision 10 cm. long, exhibiting, along its deepest parts, softening tissues infiltrated with pus. The lower portion of the manubrium sterni and part of the corpus were absent, a finger being thus readily admitted into the suppurating mediastinal tissue. The sternal end of the cartilage of the second rib on the left side was likewise wanting, while the corresponding cartilage on the right side was stripped of perichondrium.

The *head*.—Soft tissues of scalp pale. Skull of normal size and configuration. Dura mater tense, its sinuses containing fluid blood and post-mortem clot. The inner meninges pale and delicate throughout, except beneath the longitudinal sinus, where a few Pacchionian granulations were found. Moderate adhesion of the meninges to the convolutions, which latter were markedly flattened, and the whole brain swollen. Basal arteries thin-walled. Cerebral substance pale, soft, and oedematous. In the left occipital lobe were found three rounded abscesses, each about the size of a walnut and containing thick, green, fœtid pus. These, though surrounded by a definite "pyogenic" membrane, communicated with each other and occupied almost the entire white substance of the lobe, encroaching, too, over its lateral surface to the

¹ Boström, "Untersuchungen ueber die Aktinomykose des Menschen," *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1891, bd. ix.

² Bollinger, "Ueber primäre Aktinomykose des Gehirns beim Menschen," *München. med. Wchnschr.* 1887.

³ Demonstrated by Prof. Chiari, before the Verein deutscher Ärzte in Prag., Dec. 10th, 1886.

cortex. In the ventricles there was but a small quantity of serum, and elsewhere no pathological condition beyond a small localised area of intense hyperæmia on the cortex of the right middle frontal lobe.

The *pharynx*, *larynx*, and *trachea* were normal. The *teeth*, though incomplete, manifested no sign of caries; and the alveolar process, from which the lacking teeth had disappeared, showed no change other than atrophy. The *tonsils* were of normal size, their crypts containing a small quantity of mucus.

On removal of the *sternum*, not only was the area about the sinus found involved, but, further, the whole posterior surface of the manubrium sterni and corpus, as well as the sternal ends of the first three pairs of ribs, were markedly eroded, while osteophytic deposits, thickening of the periosteum, and purulent infiltration were superadded.

The *mediastinal tissue* was very dense and purulent, and penetrated by numerous sinuses running in various directions. Nor was the phlegmonous condition confined to these limits, but could readily be traced hence to the parenchyma of both *lungs* at their apices. Here the anterior and external portions were most involved, each lung consisting at this part of a dense mass of infiltrated tissue, in size equal to an orange. Both lungs in these areas were penetrated by numerous sinuses, the condition on the left side being more advanced, and presenting cavernous dilatations amid a compact mass of pigmented cicatricial tissue. Thickening of the large interlobular septa characterised the main changes in the right lung. Apart from these conditions were delicate apical adhesions of the pleura, hyperæmia of both lungs, and a moderate grade of lobular pneumonia in the right lowest lobe. The bronchi contained mucopurulent secretion; the peribronchial lymph glands somewhat enlarged and anthracotic.

In no other organ were any definite pathological lesions to be discovered. The right *tibia* and *femur* were also examined, being sawn through in a longitudinal direction, likewise the whole *vertebral column*, but in none was there any evidence of pathological change.

Microscopic examination of *fresh specimens of the pus* removed from the abscesses in the brain and lungs, as well as from the sinuses in the skin, revealed the presence of *actinomyces* in all.

PATHOLOGICAL ANATOMICAL DIAGNOSIS.—*Actinomyces pulmonum, sterni et costarum; abscessus actinomycotici metastatici cerebri lobi occipit. sin.; bronchitis suppurativa; Pneumonia lobularis lobi infer. dextri; cirrhosis hepatis grad. levioris.*

Specimens hardened in alcohol, embedded in celloidin and variously stained with magenta, orseille, hæmatoxylin, and eosin, were prepared from the affected portions of the lungs and pleura, brain, skin, and retrosternal tissue.

The sections from the *lungs and pleura* presented a condition of extensive and advanced chronic inflammation. Where the pleural surfaces were seen there was a marked increase of fibrous tissue, containing in some places but few long spindle-shaped nuclei, while in others the nuclei were more numerous, short and rounded, the latter apparently representing a more recent process. In such specimens the fibrous tissue was seen to be invaded by narrow tracts of suppuration, these containing large numbers of pus cells in a state of fragmentary and fatty degeneration. The walls of these sinuses consisted of dense fibrous tissue, which likewise was infiltrated with small round cells,

extending to a greater or less distance from the margin. The actinomycetes, which were here both large and numerous, showed distinctly the clubbed formation of their fibrils, and were surrounded on all sides by leucocytes lying within the suppurating tracts. Dense bands of fibrous tissue, dipping down from the pleura into the lung tissue, participated in the fibroid change. The interlobular as well as the interalveolar septa were markedly thickened and beset with anthracotic pigment. In the bronchi extensive changes had likewise occurred, small round cells in various stages of degeneration filling their lumina, while in many instances there was but little evidence of epithelial lining. The walls, further, were to a marked extent the seat of purulent infiltration, which could be traced to various distances into the surrounding tissue, as well as filling the alveoli themselves. In this way suppurating tracts had evidently arisen, and according as these were cut transversely or in a longitudinal direction, different pictures would be afforded — in the former case, giving the appearance of numerous minute abscesses amid fibrous tissue, in the latter case showing definitely the sinus-like tract formed by the parasite of the disease.

Sections of the *brain*, at the margin of its abscesses, showed that these, for the most part, were well defined in their boundaries, their walls, though infiltrated with small round cells, making a rapid transition to the normal brain tissue. In the surrounding cerebral substance, the vessels were dilated and filled with blood, presenting, further, collections of leucocytes in the tissues around their walls, where, likewise, there was proliferation of the connective tissue elements, and swelling of the neuroglia cells. The actinomycetes, which were readily demonstrated, were small, stained well, and existed chiefly, as in the lungs, amid the small round cells within the abscess cavity. Specimens from the *anterior thoracic wall*, cut so as to include the *sinuses*, showed conditions where masses of pus cells, surrounded by more or less dense fibrous tissue, were the prominent feature. In addition, a moderate amount of granulation tissue was present outside the abscess cavity amid the fibrous masses. Centrally situated among the pus cells were numerous actinomycetes, which, however, stained less readily than those in the brain, where apparently the process was one of much more recent date. The *retrosternal cellular tissue* presented changes of a similar nature to those in the thoracic wall, though with a greater preponderance of granulation tissue surrounding the abscess cavities. As in all the other sections, so here, large numbers of small round cells were found closely surrounding the actinomycetes, whose stage of development was apparently of a date approximating those found in the thoracic wall.

CASE 2.—J. K., æt. 16, a labourer; was admitted on June 2nd, 1892, to the medical klinik under the care of Professor Dr. Pribram. From the history there taken it may be mentioned that in November 1891 there was observed in this patient a chronic suppurative process, considered by him as spon-

taneous in origin, on the right leg to the inner side of the tibia. In the following March an analogous process manifested itself in the superficial structures on the right side of the thorax. Early in May, while driving in a carriage, patient experienced sudden palpitation of the heart, followed by a left hemiplegia; headache and occasional vomiting supervened. On admission the above history was confirmed by clinical observations: a left hemiplegia with left facial paralysis—several sinuses in the right leg to the inner side of the tibia, and on the right side of the thorax. Physical examination revealed dulness and bronchial breathing over the lower half of the right lung. The headache and vomiting still persisted. On June 10th bilateral optic neuritis was discovered, and on the following day, for the first time, rigidity of the neck muscles. Patient lost consciousness on the 14th, and died the next day at 6.45 P.M.

The CLINICAL DIAGNOSIS was as follows:—*Chronic cerebral tuberculosis of the right hemisphere, followed by basal tubercular meningitis (double optic neuritis); left hemiplegia; tubercular osteitis of the sixth and seventh ribs of right side; apical pulmonary tuberculosis.*

AUTOPSY (performed June 16th, 1892, 15 hours after death).—*Body* was that of a young man, 147 cm. long, of slender build; panniculus adiposus thin; lividity in dependent parts; rigor mortis present; pupils moderately dilated and equal; visible mucous membranes pale. The *teeth* were all present and in good condition, with the exception of the left inner incisor of the upper jaw, which was absent, while on the anterior surface of the mucous membrane of its alveolar process there was a small area of ulceration. A probe introduced at this point could be carried along a fistulous tract 1 cm. long and leading down to bared bone.

The *thorax*, of normal size and well developed; on the right side in the region about the anterior axillary line, from the sixth to the eighth ribs, were the openings of several sinuses into which a probe could be passed in various directions beneath the undermined skin. Behind, at a point slightly to the right of the eighth and ninth dorsal spines, was a similar opening about 2 cm. in diameter. On slitting up the sinus leading from it, the subcutaneous tissue was found to be involved over an area about 10 cm. in diameter, while in the muscles were numerous greyish-red nodules varying in size from a hempseed to a walnut, all presenting a softened centre and in many cases intercommunicating by suppurating tracts. At no point, however, was any connection with the vertebræ visible, nor could any of these sinuses be shown to communicate with those in the right side of the thorax. Numerous fibrous strands traversed these areas in various directions. To the inner side of the *right tibia*, along its upper half, were several other orifices situated amid pale violet coloured cicatricial tissue and leading beneath the skin; incision into the part showed the muscles on the inner side of the leg, from the knee downwards, beset with suppurating tracts running between bands of fibrous tissue. The *tibia* and *fibula* were found intact.

The *head*.—Skull mesocephalic, 50 cm. in horizontal circumference; of normal configuration; the bones thin and containing a moderate amount of diploë. The dura mater was very tense, while in the longitudinal sinus were post-mortem clots and dark fluid blood. The inner meninges were thin and delicate throughout, moderately vascular and non-adherent. The right cerebral hemisphere was much more voluminous than the left, the cortex and inner meninges about the fissure of Rolando of a greenish colour, and presenting here distinct fluctuation. Incision over this area revealed an abscess about the size of a goose egg, filled with thick greenish tenacious pus, of a remarkably fœtid odour. Around this main abscess were numerous smaller seats of suppuration, varying in size from a pea to a walnut, all containing pus of a similar character and each surrounded by a definite greyish-white capsule.

Closer examination showed that the involved areas lay in the upper third of the ascending frontal and parietal convolutions and the posterior third of the middle and superior frontal, together with a part of the corona radiata. Numerous minute ecchymoses were seen in the cerebral substance about the abscesses. The basal ganglia were free from disease and were separated from the suppurating areas by white substance, 1 cm. in thickness. Otherwise the cerebrum, cerebellum, and medulla, as well as their vessels, were in all respects normal, except that the cerebral convolutions were somewhat flattened.

The *oral cavity*, *pharynx*, and *larynx* were pale; the *tonsils* and *thyroid gland* manifested no evidence of disease. The *right lung* at its lowest portion was firmly adherent, the overlying pleura markedly thickened and traversed by numerous sinuses. From section of this part the lung was seen to be converted into a dense thickened mass of fibrous tissue in which but little trace of alveolar arrangement remained; no nodules were discernible. Elsewhere in the organ there was no sign of disease, nor did the left lung present any abnormality beyond hyperæmia and a few areas of hypostatic congestion. *Heart* and *pericardium* normal.

Peribronchial lymph glands of the right side partially enlarged, and presenting numerous greyish-white areas. *Abdomen* had no abnormal contents. *Liver* normal. *Spleen* pale but not enlarged.

Kidneys were pale, somewhat large and friable; numerous isolated greyish-white areas of the size of hempseed were dotted over the surfaces of both organs. The remaining portion of the genito-urinary tract normal in every respect. *Stomach* and *intestines* presented no evidence of disease. Likewise the *pancreas* and *adrenals*.

Examination of *fresh specimens* of the *pus* from each of the cutaneous sinuses, as well as from the cerebral abscesses, showed yellowish granules in which the actinomyces were readily detected. Numerous and careful examinations, however, failed to reveal, in fresh specimens, any sign of the same germ in the contents of the ulcer situated about the alveolar process of the upper jaw.

PATHOLOGICAL ANATOMICAL DIAGNOSIS.—*Actinomyces pulmonis d. Actinomyces parietis thoracis. Abscessus actinomycotici metastatici cruris d., cerebri hemisphæris d. et renum.*

Hardened specimens from affected portions of the right lung, brain, skin, and kidneys as well as from the tonsils and peribronchial lymph glands, and the diseased part of the upper jaw, were embedded in celloidin. From these, numerous sections were cut and stained with hæmatoxylin, as well as after Gram's method.

The sections from the lungs manifested conditions of both acute and advanced chronic inflammation. In the former case were seen numerous abscesses and tracts of suppuration in various portions of the lung tissue. The bronchi especially were affected, showing collections of leucocytes in their lumina as well as all about their walls, from which the epithelial lining had in great part disappeared. Directly in connection with these bronchi and bronchioles could be seen many of the above mentioned suppurating tracts, leading in various directions into the surrounding tissue. In numerous specimens, too, actinomyces were found directly in the lumen of a bronchus, whose epithelial lining was often readily seen. The parasites here were large, partially calcified, stained rather poorly, and showed numerous clubbed fibrils. The chronic inflammation, on the other hand, was manifested by advanced interstitial pneumonia,

the alveolar arrangement of the lung tissue being scarcely discernible; while in those areas, where the alveoli had retained their outline, their lumina were filled with small round cells, and the walls were greatly thickened. The presence of thickened arteries, and marked anthracosis was superadded. Specimens of the *brain* were examined both from the margin of the larger abscesses and from where the minuter seats of suppuration existed. The larger abscesses presented at their edges dense masses of small round cells closely packed together, and evidently in a stage of advanced degeneration. Around this area was a zone of concentrically arranged fibrous tissue of recent growth, which in most places gave a fairly definite boundary to the abscess, though in other parts the limitations were less distinctly defined, inasmuch as a moderately copious small cell infiltration invaded the surrounding cerebral tissue.

The cerebral vessels in the neighbourhood were somewhat thickened, dilated, and surrounded by a greater or less number of leucocytes. The actinomycetes, in moderate quantity, were small, stained deeply, and existed only amid the dense masses of cells in the abscess margin. Sections from the brain, cut so as to include the minuter abscesses, presented, in these, collections of leucocytes surrounded by recently formed fibrous tissue with many nuclei. The surrounding cerebral substance was infiltrated with small round cells, and contained vessels filled with blood, around whose walls were numerous leucocytes. In no place, however, was there any evidence of true chronic inflammation. The actinomycetes were here present in large numbers, and centrally situated in the abscesses. In all cases they stained well, the filaments being particularly distinct, and the clubbed ends in most cases wanting.

The *kidneys*, too, were examined, and the nature of the small greyish white areas more accurately determined. These in the form of minute multiple abscesses, chiefly in the cortex, existed in isolated patches, while the kidney substance presented otherwise merely a parenchymatous degeneration of its tubular epithelium and numerous casts. No general small-celled infiltration nor granulation tissue could be detected. The abscesses consisted merely of circumscribed masses of small round cells, containing in some instances giant cell formations. The invading parasite was likewise found in the centre of one of these abscesses in the immediate neighbourhood of a glomerulus, being in an early stage of development, composed chiefly of filaments, and manifesting but little evidence of clubbed formation.

The *skin*, examined by similar methods, showed in its sinuses numerous broken-down leucocytes, and the edges likewise infiltrated with pus cells. The walls of the sinuses contained more or less recent fibrous tissue, with small-celled infiltration, and the vessels of the neighbouring parts were hyperæmic, and surrounded by clusters of leucocytes. Here the actinomycetes were found in moderate numbers among the broken-down pus cells within the sinuses, and their filaments showed with

marked clearness numerous clubbed endings. The *tonsils* and *peri-bronchial glands* contained no evidence of infection, while careful examination of sections of the ulcerated alveolar process showed, beyond various stages of osseous inflammation and caries of the dental root, no trace whatever of actinomyces. Sections specially stained by Gram's method and with methylene-blue showed no evidence of other micro-organisms in any of the tissues examined. There too, however, in addition to the small leucocytes, were seen several giant cells, in most cases observed in the centre of a glomerulus, while the parasite was seen centrally situated in an abscess, immediately in juxtaposition to the capillary loops.

As regards the sinuses in the skin and subcutaneous tissue, those in the right leg are likewise undoubtedly metastatic, whereas all those in the side of the thorax and in the back, although also probably metastatic, cannot be positively regarded as such, inasmuch as their intimate topographical relationship to the diseased lung might render infection by continuity a possibility. The fact that no direct connection between the two processes could be found, does not exclude this means of infection, inasmuch as the parasite, in many instances, may penetrate extensive areas of tissue without a noticeable trace of its advance being left behind.

Finally, from the facts above given, one will observe the very marked resemblance of the disease in Case 1, to the tubercular process, inasmuch as here a distinctly atypical course was taken, viz., an involvement of the pulmonary apices. Its importance to the clinician may make it worthy of mention in view of the fact that J. Israel, in his remarks on differential diagnosis, regards the basal seat of the actinomycotic lesions as one of the main criteria in distinguishing that disease from tuberculosis.

From the above data it will be apparent that an inquiry into the pathogenesis in these two cases presents no great difficulties, and that, in both instances, the nature of the process warrants their being placed under Group 2 of J. Israel's classification, namely, "primary actinomycosis of the lungs." In Case 1 the very chronic pulmonary inflammation with its extreme fibroid changes, especially in the left lung, where the conditions were more advanced, represents, without a doubt, the oldest pathological process in any of the organs. Further, the condition of the smaller bronchi, whose lumina filled with leucocytes were continuous with suppurating tracts, traceable to varying distances in the altered lung tissue, and in which the parasites were readily found, render it all the more certain that here the original invasion took place. Hence, the disease progressed *per continuitatem* towards the periphery, affecting the overlying pleura, and reaching the mediastinal tissue till it finally, after eroding the bones in its course, perforated the anterior thoracic wall. In the brain the lesions were undoubtedly metastatic in origin, arising

from invasion of the systemic circulation by the parasite. Microscopically, these metastases presented the ordinary appearances of abscesses.

The second case affords still more clearly a ready explanation of the parasite's invasion and progress within the body. That the disease was likewise pulmonary in origin is proved from the fact that, not only were actinomyces found in the suppurating tracts leading from the bronchi, but directly within the lumina themselves. Moreover, the extensive growth of interstitial tissue likewise points to the lungs as the seat of the oldest pathological affection. Invasion, then, of the lungs was succeeded by a markedly slow, chronic, and insidious process, to which no attention was drawn, till after a metastatic area had been discovered in a distant part of the body. Having, then, slowly progressed in the lung tissue, the disease was here propagated directly on to the overlying pleura, which likewise became chronically inflamed and formed dense adhesions. From the original seat of invasion, metastases, through the blood channels, invaded the brain and kidneys. Those in the brain presented features resembling very closely the conditions described in Case 1, there being but little granulation tissue present, and the main mass consisting of broken-down leucocytes, not differing therefore in general appearance from the usual form of pyæmic metastases.

By far the most recent process was seen in the kidneys, where, too, the connection of the infection with the circulatory system was evident.

THERMOPHILIC BACTERIA.

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THE range of temperature at which it is possible for bacteria to grow is very great. Forster,¹ for example, proved that certain bacteria were capable of growth and multiplication at zero. These organisms were found by him in water, milk, soil, and street mud. When cultivated at this low temperature they still produced pigment, gas, light, etc. The apparently unfavourable external conditions did not overcome the vitality of the cells or interfere with the exercise of their specific properties.

For a large number of bacteria, 15°–20° C. is the optimum temperature, whilst for pathogenic bacteria the optimum temperature is blood heat. At temperatures above 40° C. relatively few micro-organisms grow, and at 50° C. most nonspore-bearing organisms quickly die. But there is undoubtedly a group of micro-organisms which can grow at temperatures above 50° C., and for which such high temperatures furnish the best conditions for their growth and development. Miquel's *Bacillus thermophilus*² is perhaps the most familiar example. It was found by him in air, rain water, river water, and in the alimentary canal of man and animals. It grows best at a temperature of from 65°–70° C.

Van Tieghem³ also describes a micrococcus which grew at 70° C., and Certes and Garrigon⁴ found, in the waters of Luchon, bacilli that could be cultivated at 64° C. The temperatures at which it is possible for bacteria to grow range therefore from zero to 70° C. Globig,⁵ on inoculating sterile milk and beef-broth with garden earth, obtained a growth of bacteria at 58° C. They were exclusively bacilli, and in many cases they produced spores after 24 hours' incubation at this temperature, potatoes being used as a culture soil. Globig obtained 30 bacteria, which grew at 58° C. At 68° C. only a few continued to grow. One bacillus grew at 68° C. and also at 15°–20° C. This was the greatest range of

¹ *Centralbl. f. Bakteriöl. u. Parasitenk.* bd. xii.

² *Ann. de Micrographie*, No. 1, 1888.

³ *Bull. Soc. Bot. de France*, tome xxviii.

⁴ *Compt. rend. Acad. sc.*, Paris, tome iii.

⁵ *Ztschr. f. Hyg.*, Leipzig, bd. iii.

temperature observed by Globig. As a rule, growth only began about 50° C. The bacilli were all isolated from garden soil. Experiments made with fæces and with water from various sources gave negative results. Globig, therefore, draws the conclusion that the thermophilic bacteria found by him in garden soil are not derived from the alimentary canals of animals or from water.

These are the main researches that we have been able to find on this subject, and they are more or less fragmentary. Beyond the fact of demonstrating their existence, comparatively little has been done in the study of this interesting group of organisms as a whole.

We undertook this investigation in order to determine whether the bacteria which grow at such high temperatures are widely distributed, and their number considerable; whether, in other words, they are to be looked upon as mere bacteriological curiosities, or as organisms which, from their number and distribution, probably fulfil some function in the economy of nature. We will first state what we have been able to determine with regard to their morphology, and finally touch upon their salient biological properties. To those organisms that grow best at very high temperatures we have applied the name of thermophilic bacteria, and this term will be employed in the course of this paper. The temperature we used in our experiments was attained in an incubator set at 60°–65° C., a temperature far removed from that at which the ordinary saprophytic bacteria will grow. By this means we avoided the introduction of any possible fallacy. If bacteria grew well at this high temperature, there would be no doubt about their being genuine thermophilic organisms.

The suggestion occurred that perhaps the spores of thermophilic organisms might be present in our culture soils, and that owing to their great resistance to heat they might survive the ordinary methods of sterilisation. We accordingly placed tubes containing sterilised gelatine, agar-agar, broth, potato and milk, in an incubator at 60°–65° C. Though the tubes were left for some days at this temperature, no growth appeared, nor did the milk curdle. We thus demonstrated, in the first instance, that our culture soils were free from thermophilic bacteria, and that they had been rendered absolutely sterile by the usual methods employed.

Control cultures were also made of well-known saprophytic bacteria, to be met with in various soils at ordinary temperatures. We selected *Proteus vulgaris*, *Proteus mirabilis*, *Proteus zenkeri*, *B. mesentericus fuscus*, *B. subtilis*, *B. mycoides*, and the *B. fluorescens liquefaciens*. These organisms showed no trace of growth, though left for 16 days in an incubator at 63°–65° C. We were thus able to settle two important points at the outset, viz. that our nutrient culture media were free from thermophilic bacteria or their spores, and that the thermophilic bacteria were organisms differing from those commonly met with in various soils at ordinary temperatures.

The first experiments were made with garden soil. We found that after inoculating nutrient agar-agar with garden soil, an abundant growth of bacteria was invariably obtained at 60°–65° C.

We endeavoured to determine, in the first place, whether these thermophilic bacteria are widely distributed in nature. The culture soils used for this purpose were gelatine, agar-agar, beef-broth, Koch's peptone water, and potatoes. We selected a variety of nutrient soils, in order to obtain as large a number of organisms as possible. Tubes containing these several culture media were inoculated directly with material from various sources. The tubes were then placed in an incubator at 60°–65° C. The results, concisely stated, were as follows:—

1. EXPERIMENTS WITH FÆCAL MATTER.

For the experiments, human fæces, mouse fæces, hen fæces, and horse dung were used. In all cases the results were positive. We obtained a good growth of bacteria at 60°–65° C. A sample of dysenteric fæces, which was sent to the laboratory, was also tested, and a growth of bacteria likewise obtained at the above temperature. It may be mentioned here that human saliva gave negative results.

2. EXPERIMENTS WITH SEWAGE MATTER.

We used for this purpose samples of Croydon sewage, before its passage to the sewage farm. Good growths of bacteria were obtained in the various culture media at 60°–65° C. The effluent, after leaving the sewage farm, was also found to contain thermophilic bacteria.

3. EXPERIMENTS WITH SAMPLES OF SOIL.

The surface soil taken from a London garden was found to contain numbers of thermophilic bacteria. Further, samples of soil taken at a depth of 2 in., 15 in., and 2 ft., gave in each case a growth of bacteria at 60°–65° C. We thus found those thermophilic organisms at a depth of 2 ft.

In a made London soil, owing to frequent disturbance, there must have been a considerable mixing of the deep and surface layers. We therefore made a series of experiments in a London suburb, in a garden where the soil had been undisturbed for years.

Samples of this undisturbed soil were taken from various depths by means of a Fränkel's earth borer. We examined the soil at a depth of 5 ft., 4 ft., 3 ft., 2 ft., and 1 ft., as well as the surface layers. All these samples gave a growth of bacteria at 60°–65° C.

The thermophilic bacteria are therefore not only widely distributed in the surface layers of the soil, but are to be found in the soil even at a depth of 5 ft.

4. EXPERIMENTS WITH SAMPLES OF WATER.

Thames water invariably gave a good growth of bacteria at 60°–65° C.

Thames mud taken from the bed of the river also gave a copious growth at the same temperature.

A sample of *sea water* taken at Broadstairs gave a positive result.

The results with London tap water were negative. The water from a thermal spring at Bath also gave a negative result. We were, however, only able to examine one sample of this water.

5. EXPERIMENTS WITH AIR DUST.

Rain which was collected during a shower gave no growth of bacteria at 60°–65° C.

The dust collected from the floor of the laboratory was found to contain thermophilic organisms, but the dust collected near the ceiling gave no growth.

The dust gathered from the London streets gave a good growth in every instance.

Finally, thermophilic organisms were also obtained from straw which had been placed in beef-broth, and incubated at 60°–65° C.

These thermophilic organisms are, therefore, most widely distributed—we found them in water, the soil, and air dust; in river mud, straw, and the dejecta of man and the lower animals.

Their distribution is wide, and they may almost be termed ubiquitous, as our experiments show.

It is interesting to note that these thermophilic organisms are not confined to the surface of the earth, as one would naturally expect, but that they extend to a considerable depth in the soil. We found them in the mud from the bed of the Thames, and in soil at a depth of 5 feet. Another point of interest is their very rapid growth at 60°–65° C. Agar tube cultures, placed in an incubator at 60°–65° C., gave a rapid and extensive growth in 15–17 hours, showing that this high temperature was one most favourable to the growth and development of these organisms.

The above experiments will be sufficient to show that these thermophilic bacteria are widely distributed, and that they are capable of existing under the most varied external conditions.

Our next endeavour was to isolate these organisms in pure cultures. The method we adopted was as follows. Nutrient agar-agar was liquefied, poured into Petri's culture dishes, and allowed to resolidify. An emulsion of the material to be investigated was made in distilled water. By means of a camel-hair brush, some of the emulsion was smeared over the surface of the agar-agar so as to form a very thin film. The culture dishes were then inverted, in order to prevent moisture running over the surface of the agar. The cultures so prepared were placed in a glass vessel, and covered with a bell jar. In the bottom of the glass receptacle some water was placed to prevent the agar becoming too dry. The vessel containing the plate cultures was placed in an incubator at 60°–65° C. In this way colonies of the thermophilic bacteria were obtained. From the colonies subcultures were made on various media, and their manner of growth was studied. The colonies developed on the plates very rapidly. After from 15–17 hours, good growths were generally obtained.

We may state here that all the thermophilic organisms examined by us were bacilli. We did not obtain any moulds, yeasts, cocci, spirilla,

or cladothrix forms. Further, all the organisms isolated by means of plate cultures, proved to be bacilli. We were also able to demonstrate that these bacilli do not all belong to one species, but that there are quite a variety of thermophilic bacilli. Representative forms, isolated from different sources were taken, and their morphology and growth on various culture media more closely studied.

In this way we were able to differentiate at least twenty different species. The number might easily have been extended, if we had carried our investigations further in this direction. The appended table will, however, suffice to give an idea of the variety of these forms that exist widely distributed in nature.

A bacillus, which was isolated from sea water, showed a very characteristic serpentine movement, which we only noticed in this form. A bacillus isolated from Thames mud produced a characteristic brick-red pigment on nutrient agar-agar.

As already stated, *all* the organisms isolated by us were bacilli.

The classical example of a thermophilic organism has hitherto been Miquel's *Bacillus thermophilus*. It might perhaps therefore be natural to assume that the number of bacilli capable of development at such high temperatures is very limited. Our experiments, however, speedily showed that there are quite a number of bacilli to which the term "thermophilus" may be employed. The twenty forms isolated by us did not exhaust the number. We must, therefore, regard the thermophilic bacteria as a large and important group of micro-organisms. Our observations quite confirmed those of Globig, as to the variety of species of the thermophilic bacilli. All the organisms that we carefully examined were spore-forming bacilli. We could distinguish four groups of bacilli, as regards spore formation:—

1. Those in which the spore is formed at or near the centre of the bacillus.
2. Those in which a round spore is formed at one end of the bacillus, giving the "drumstick" appearance.
3. Those with a large oval spore at one end of the bacillus.
4. Those with a small spore, not exceeding the diameter of the bacillus, at one end.

The spore formation took place at 60°–65° C., and the best media to study the production of spores were surface agar-agar and potato cultures.

A further point of interest was the motility of a number of the organisms. In hanging-drop specimens a number showed active movements, darting about with great rapidity. It was interesting also to note how long this motility lasted. A culture kept for three weeks at 60°–65° C., still contained actively motile bacilli—a further indication of the suitability of this temperature for the organisms. There was further a marked tendency to the formation of long chains of bacilli. These were often of great length, and stretched across the greater part of the field of the microscope. The chains were either straight, curved, or twisted

TABLE I.
Bacilli found in Thames Water.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar-Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Very long chains; ends round; end spore; motile.	Diffuse, greyish-white growth.	Slight surface growth.	Flocculent, greyish-white growth.	Cloudy, diffuse growth.	Cream-coloured growth.	No precipitation of casein.
2	Short chains; ends round; end spore; motile.	Diffuse, dull white growth.	Very slight growth.	Ropy, viscous growth; liquefaction.	Very faint growth.	Little or no growth.	No precipitation of casein.
3	Curved chains; ends round; central spore; motile.	Cream-coloured, diffuse growth.	Do.	Diffuse growth.	General turbidity.	Yellowish-brown growth.	No precipitation of casein.

TABLE II.
Bacilli found in Surface Soil.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Long, delicate threads; central spore; motile.	No growth.	Slight surface growth.	No growth.	Surface pellicle.	Very slow growth; amber coloured.	No precipitation of the casein.
2	Short threads; central spore; motile.	Small dots; then diffuse.	Do.	Do.	Turbidity; no pellicle.	Yellowish, glistering growth.	No precipitation of casein.
3	Long threads; end spore; non-motile.	White, diffuse growth.	Do.	Liquefaction.	Do.	Whitish film.	Curdles milk; precipitation of casein.
4	Long, slender threads; end spore; non-motile.	Whitish growth.	Do.	No liquefaction.	Do.	Yellowish-white.	Curdled.
5	Slender threads; central spore; non-motile.	White, granular growth.	Do.	Liquefaction.	Do.	Brownish-yellow.	Curdled, precipitation of casein; pink pigment.
6	Short, straight rods; rounded ends; end spore; motile.	Diffuse, dull white growth.	Very slight growth.	Pink, flocculent growth; liquefaction.	Diffuse cloudiness.	Brown, glistering growth.	Curdled.
7	Plump bacillus; round ends; non-motile; central spore.	White, round patches.	Do.	Liquefaction.	Diffuse growth and pink pellicle.	Pale pink.	Do.
8	Short, straight rod; end spores; non-motile.	Slimy, diffuse growth.	Do.	Flocculent growth; liquefaction.	Diffuse cloudiness.	Feeble growth; yellow pigment.	No action.

TABLE III.

Bacilli found in Street Dust.

No. of Bacillus.	Form and Arrangement.	Growth on Agar-Agar.	Growth on Sugar Agar.	Growth on Gelatine.	Growth in Broth.	Growth on Potato.	Growth in Milk.
1	Short rods; end spores; non-motile.	White film.	Not noted.	Slight, cloudy growth.	Diffuse cloudiness.	Honey-like growth.	Not noted.
2	Long, slender threads; end spores; motile.	Whitish film.	Do.	Turbidity; liquefaction.	Turbidity.	Transparent, whitish, shiny growth.	Curdled.
3	Short rods; end spores; motile.	No growth.	Do.	Turbidity; no liquefaction.	Diffuse cloudiness.	White crust.	Do.
4	Thin, slender rods; sometimes long threads; end spores; non-motile.	Arborescent growth.	Do.	Turbidity; liquefaction.	Do.	Slow, pink growth, with white edges.	Do.

The organisms were incubated at 60°-65° C. The gelatine was, of course, liquefied at these temperatures. Liquefaction of the gelatine was determined by removing the tubes to room temperature after a sufficient period of growth, and controlled by uninoculated gelatine, subjected for the same period to the same temperature.

in shape. There was no difficulty in staining the bacilli with the ordinary aniline dyes, nor in staining the spores by the usual methods. With regard to their growth on culture media, the ordinary soils gave good growths. Individual forms developed well on gelatine, agar, broth, potato, peptone water, and milk. The quickest growth was generally obtained by means of surface agar cultures. Differentiations in pigment production were most markedly developed in potato cultures—though the growth of the organisms was slower than on agar.

A certain number of the bacilli liquefied gelatine, some curdled milk, and others were specially characterised by the production of pigments. After having obtained pure cultures of the bacteria, it was of importance to prove their thermophilic properties. The term "thermophilic," strictly speaking, implies an organism that grows best at high temperatures; that can be cultivated from generation to generation at such high temperatures; and that does not grow, or at most feebly, at low temperatures. This test was applied to the bacilli we had isolated.

Subcultures were made, and placed first at 22° C., then at 37° C., and finally at 60°–65° C. No growth took place at 22° C., nor at 37° C., but on placing the tubes at 60°–65° C. a rapid growth once more took place. These experiments seemed clearly to prove that at or below blood heat the organisms did not develop, that temperatures above blood heat were necessary for their growth, and that 60°–65° C. was an optimum temperature. Also cultures that were carried on to the sixth generation continued to grow well at 65° C. The organisms could therefore with justice be called obligatory thermophilic bacteria.

A further series of experiments was made to determine the range of temperature at which it is possible for these bacteria to grow. For this purpose the temperature of an incubator was gradually raised from blood heat upwards. Fifteen organisms from various sources were tested. At blood heat no visible growth took place. One organism (a bacillus isolated from horse dung) began to grow at 40°–42° C. A slow growth began in 6 of the tubes at 50°–52° C. A slow growth started in 2 other tubes at 52°–55° C., and in two more instances commenced at 56° C. Four cultures remained undeveloped at 56° C., and their growth first began at 60° C. None of the organisms would grow at 75° C. Whilst we obtained a growth in some instances at 50°–52° C., the growth was slower, and not nearly so abundant as at 60°–65° C. At this latter temperature an almost instantaneous growth was obtained, and it evidently furnished the best conditions with regard to heat for the bacilli. The lower limit of growth for nearly all the bacilli appears to be 50° C., and the upper limit for the forms we isolated, 75° C. They, however, developed best between 55° and 65° C., giving a range of 10 degrees within which their growth was active. The organisms possess also a considerable resistance to the action of heat, as boiling for 10 minutes did not destroy them. The temperature at which it is possible for the thermo-

philic bacteria to grow lies so far above blood heat that we did not feel it necessary to test their pathogenic properties. The organisms isolated by Globig were found to be non-pathogenic. The thermophilic organisms are undoubtedly saprophytic organisms.

Micrococci were not detected, though Van Tieghem states that he found a coccus capable of development at 70° C. Miquel's *B. thermophilus* grew in broth at 60°–70° C., but did not grow in nutrient gelatine. The thermophilic bacteria isolated by Globig from garden soil began to grow, as a general rule, about 50° C. He found them in garden earth, but *not* in the dejecta of man, dogs, guinea-pigs, rabbits, pigeons, or mice. Horse dung, sewage water, Spree water, tap water, etc., gave negative results; and he concludes that the thermophilic bacteria of the soil are not derived from the alimentary tract of animals. Our experience was different. We found the organisms widely distributed in the soil; in the dejecta of man and the lower animals, and in water from various sources. On examining a virgin, sandy soil, Globig found that these bacteria disappeared at a depth of 8 inches. In our experiments we found them as deep as 5 feet in the soil. Lustig¹ describes a "red bacillus" which grew at room temperature, and also at 60° C., having therefore a remarkably wide range of growth. This bacillus produced no pigment when cultivated above blood heat. It is motile, and produces no endogenous spores. The bacillus reduces nitrates and develops nitrous acid. It, however, seems to be a rare form, as it was only isolated once from river water by Lustig.

There still remain some points of interest to be noted in connection with the general biology of the thermophilic bacteria we isolated. The inquiries made in this direction were by no means of an exhaustive nature; but sufficient indications were obtained that a more extended chemical investigation would lead to valuable and important results. The directions in which such investigations might be fruitful, will be sufficiently indicated by the following summary of our results.

A certain number of the bacilli liquefied gelatine, others did not. A certain number also produced a curdling of milk. A putrefactive decomposition took place in broth and agar cultures, characterised by a most repulsive smell, and the production of indol and sulphuretted hydrogen. The action of four organisms was tested on starch. Three of these produced no change in the starch. One bacillus (isolated from mouse faeces) converted the starch into sugar. The organisms did not produce any fermentation of sugar, nor did they thrive on soils containing sugar. Their growth on a sugar soil was very slow. It was evident that the presence of sugar did not favour, but was inimical to their growth. A good growth was obtained on urine in several instances. A bacillus isolated from horse dung produced a marked putrefactive decomposition of the urine. Cultures made in eggs gave, in one or two instances, H₂S gas.

¹ "Diagnostik d. Bakter. d. Wassers." 1893, p. 72.

Experiments were made with meat and with blood albumen. To one part of meat, four parts of water were added, and the whole sterilised in a flask. The blood albumen was prepared in the same way. The flasks were then inoculated with a small quantity of Thames mud, or of surface soil, and placed in the incubator at 60°–65° C. An active decomposition of the proteid matter took place, and a great development of H_2S , accompanied by an overpowering, nauseating odour. Indeed the smells developed on proteid soils by these thermophilic bacteria are things to be remembered.

A certain number produced pigments at these high temperatures, *e.g.* pink, yellow, or brown. A bacillus from Thames mud produced a brick-red pigment on agar-agar. The action of the organisms on cellulose was finally tested. A little meat broth or salt solution was added to straw or to cotton wool. The tubes were then inoculated with the micro-organisms. A bacillus from the soil, and one from Thames mud, grew well under these conditions, and gas was developed. Whilst not wishing to lay too great stress on these comparatively simple experiments, they sufficiently indicated that certain of the organisms, under favourable conditions, can probably induce a fermentative decomposition of cellulose. We may also add that straw suspended in broth gave a growth of bacteria at 60°–65° C.

Of four organisms tested, two grew anaerobically, and one of these produced gas. The anaerobic growth was, however, slow compared with the rapid, extensive surface growth of aerobic cultures of the same organism. Anaerobic sugar cultures gave no fermentation of the sugar in the cases tested, and practically no growth of the bacteria. The sugar had an inimical action on their growth under both aerobic and anaerobic conditions.

These are the salient features of our investigation, succinctly stated.

We have then existing in nature a large number of bacteria, for which 60°–65° C. is an optimum temperature, and these organisms will not grow at or below blood heat. They grow best at 60°–65° C., and continue to grow at this temperature from generation to generation. At this high temperature they exercise perfectly all the functions common to saprophytic organisms. They liquefy gelatine, curdle milk, produce pigment, and convert starch into sugar. They produce an active decomposition of proteids, characterised by the development of H_2S , and a most repulsive smell; and they probably can produce a fermentation of cellulose. Their most marked property appears to be the decomposition of proteid bodies which they are able to effect.

It is indeed remarkable to find cell protoplasm existing and exercising all its functions at a temperature which is usually fatal to its life. One question naturally arises, and it is by no means an easy one to answer. How do these organisms exist under the conditions of temperature prevailing in this part of the globe? In the tropics the surface soil becomes greatly heated by the sun, and travellers tell us

that an egg can be cooked in the sand of the Sahara Desert. A badly conducting surface like sand becomes very hot when exposed to the sun's rays, and a dark surface soil absorbs a large quantity of heat. A superficial temperature of $68^{\circ}3$ C. has been observed in a sandy soil at the Cape of Good Hope. At Edinburgh, in a hole bored 2 feet into the solid rock, the minimum temperature was $4^{\circ}1$ C., and the maximum $11^{\circ}6$ C., giving a range of $7^{\circ}5$ C. for the year.

The conditions of climate existing in this country are not favourable to the growth of these thermophilic bacteria, and yet they are most widely distributed. It is apparent contradictions of this kind that stimulate investigation, and lead to valuable additions to our knowledge. Apart from climatic influences, it is most probable that these bacteria do find in nature conditions under which they are able to grow and develop. In this connection there is one point that seems to us well worthy of investigation, *i.e.*, the temperatures at which various fermentations take place, and the internal heat that is developed in the course of such fermentations. The maximum amount of nitrification of ammonia and its salts in the soil takes place at 37° C., and ceases at 55° C. We are familiar with the smoking of a manure heap. Manure, through the action of bacteria, undergoes a variety of fermentations, in the course of which considerable heat is developed. We have the most varied fermentations constantly going on in manure:—

1. The decomposition of fatty acids.
2. The decomposition of amido compounds, such as leucin and tyrosin.
3. The putrefaction of proteids.
4. The ammoniacal decomposition of urea.
5. The fermentations associated with the development of H_2S .
6. The cellulose fermentation. This fermentation occurs in every manure heap, and also in moist marshy soils. Tappeiner also found that bacteria contained in the intestines of cattle produced a cellulose fermentation, with a development of CO_2 and marsh gas. Very little is known about these bacteria. Schlosing found that he could start a very active fermentation of cellulose in manure by heating up to 42° and 52° C.¹ This cellulose fermentation is favoured by a high temperature, and is most active in summer.
7. The butyric acid fermentation, which takes place best at 40° C.

All these fermentations occur in manure, and the manure becomes sensibly hot. It may be that in the course of such fermentations, sufficient heat may be developed to start, at any rate, the growth of these bacteria.

When hay is packed moist, fermentation may occur, and so great a heat is developed within the stack that the hay may catch fire. The changes which go on in a moist haystack are also seen in the process of ensilage. If green fodder is stored in a silo, the mass becomes hot

¹ *Compt rend. Acad. d. sc.*, Paris, 1889.

from oxidation. Carbonic acid and other gases are developed. Mr. Warrington states that the temperature of the silo goes up to at least 140°–160° F. (60°–71° C.).

Ferdinand Cohn,¹ as the result of his investigations, comes to the conclusion that the "spontaneous combustion" of moist hay, etc., is due to a fermentation which is set up by micro-organisms. He found that on moistening cotton-wool waste the temperature rose, and reached in 24–30 hours a maximum of 67°·2 C. The temperature then slowly fell within 6 days to the temperature of the air. A strong odour of trimethylamine was at the same time developed. Trimethylamine is developed frequently in the course of various bacterial fermentations. Cohn believes that the cause of this fermentation of the cotton-wool waste is a micrococcus. In any case the *sterilised* cotton-wool did not ferment, nor was there any rise of temperature. In the course of the fermentation oxygen is used up and much CO₂ gas is developed. If the supply of oxygen is cut off, no rise in temperature takes place. The process is, therefore, due to the action of *aërobic* bacteria, the germs of which are present in the cotton-wool, when imported from America. The bacteria, therefore, probably belong to the large and important group of soil organisms. To these organisms Cohn applies the name, "thermogenic bacteria."

In the Augsburg hothouses the flower-pots are heated by means of this cotton waste, which is packed round them. On moistening the cotton-wool a heat is developed sufficient for the growth of the plants.

The heat, therefore, developed in the course of various fermentations is great, going up to at least 160° F. (71° C.) and even higher, *i.e.* a temperature at which thermophilic bacteria would grow very well. It would be most valuable to have accurate data regarding the *internal* or localised heat developed in the course of various fermentations that are constantly taking place on a large scale in nature. As already pointed out, it is in many cases so considerable, that thermophilic bacteria may find, under such circumstances, conditions favouring not only their growth, but also the exercise of their special fermentative properties. It may be that they are also agents in the fermentation of cellulose, and the subject deserves further investigation, as well as the investigation of the amount of heat developed in this and other fermentations.

Whilst 55°–65° C. is the most favourable range of heat for these organisms, the possibility is not excluded that in some instances, at anyrate, their growth may be started at 40°–42° C.

It is difficult to believe that these thermophilic bacteria are simply "freaks," and that they only develop when a bacteriologist appears on the scene with an incubator. Their wide distribution, their good growth at these high temperatures, and their active fermentative properties, all point to their fulfilling some useful function in the economy of nature.

¹ *Berichte d. deutsch. botan. Ges.* 1893, bd. xi.

ON THE PIGMENTATION OF URIC ACID CRYSTALS DEPOSITED FROM URINE.

By ARCHIBALD E. GARROD, M.A., M.D. (Oxon.), F.R.C.P.

(PLATE III.)

THERE is no more familiar fact of clinical chemistry than the coloration of uric acid crystals deposited from urine, but the nature of the pigment or pigments to which the crystals of this colourless acid owe their tints has never, as far as I am aware, formed the subject of a systematic study.

It is true that Wetzlar,¹ Duvernoy,² and others, extracted the colouring matter from such sediments by means of boiling water or alcohol, and examined some of its properties, but, at the time they did so, next to nothing was known about the urinary pigments, and consequently their results threw but little light upon the question. Kunkel³ has detected the presence of iron in sediments of uric acid thrown down by the addition of hydrochloric acid to urine, and regards it as a constituent of the included colouring matter; but, although the correctness of the observation cannot be questioned, there are, as I shall show, reasons for doubting the interpretation put upon it. Most modern authors who refer to the subject offer no suggestion at all as to the nature of the contained pigment, confining themselves to a description of the appearance of the crystals; but in a few works we meet with the statement that the sediments owe their colour to uroerythrin, the pigment of pink urates.

From the point of view of their pigmentation, deposits of uric acid may be conveniently classified into four groups, as follows:—

1. "Cayenne pepper" deposits, which are the commonest, and which, when seen in bulk, have a more or less intense red colour. They are composed of crystals, which, examined in detail, have a warm orange tint, occasionally verging upon red.

2. Yellow or fawn coloured deposits, consisting of yellow crystals which often show a tinge of brown.

¹ "Beiträge zur Kenntniss des menschlichen Harns," etc. Frankfort-on-the-Main, 1821.

² "Chemisch-medicinische Untersuchungen über den menschlichen Urin." Stuttgart, 1835.

³ *Sitzungsab. d. phys.-med. Gesellsch. zu Würzb.* 1881, p. 69.

3. Deposits coloured by abnormal pigments occasionally present in urine, which appear brown or black when seen in bulk.

4. Brown deposits thrown down by the addition of mineral acids to urine.

It is obvious that no single colouring matter can produce all the tints observed even in the spontaneously deposited crystals, and it is hardly possible to doubt that they owe their ground tint to the substance, whatever be its nature, which gives the normal urine its yellow colour.

The influence of the contained pigments is not confined to the mere tinting of the crystals, for they also appear to play a very important part in determining their form. Duvernoy clearly recognised this fact, which has been more recently studied by Ord¹ as an example of the influence of colloids upon crystalline form. After repeatedly redissolving the urinary crystals in water, Dr. Ord ultimately obtained specimens which were colourless, and had the tabular forms of crystals of pure uric acid.

Doubtless there are other factors simultaneously at work in moulding the crystals into the almost innumerable shapes which they assume; and of these factors the degree of acidity of the liquid is, as Sansom² has shown, one of the most potent.

Sir William Roberts³ attributes to the pigments yet another important action, namely, that of retarding the separation of free uric acid from solutions of the quadrurates.

The line of investigation which throws most light upon the question of coloration is the precipitation of crystals of uric acid from solutions containing the several urinary pigments in as pure condition as possible, and I propose to speak first of the results of a series of experiments of this kind.

The solutions of urate employed were obtained from dry and clean snake's urine, which, when treated with hot water, yields, as Sir William Roberts has shown, a solution of quadrurates from which colourless crystals of uric acid are in a short time deposited. As a rule, this material was treated with a hot aqueous solution of neutral sodium phosphate, and to the solution so obtained, after it had been freely diluted with water, a small quantity of acid sodium phosphate was added. When rapidly deposited from such solutions the colourless crystals of uric acid have the form of very thin rectangular plates, but when more slowly formed they appear as more massive square or oblong tables, or as derivatives of such forms. (Plate III. Fig. 1.)

Numerous experiments were made with uroerythrin, which was obtained in a state of tolerable purity, but not entirely free from yellow pigment, by a method described by Riva,⁴ which consists in

¹ "The Influence of Colloids upon Crystalline Form and Cohesion," 1879, p. 52.

² Quoted by Beale, "Kidney Diseases and Urinary Deposits," 3rd edition, 1869, p. 371.

³ "Croonian Lectures on Uric Acid, Gravel, and Gout," 1892, p. 46.

⁴ *Gazz. med. di Torino*, 1892, vol. xliii. p. 3.

washing pink urate sediments with iced water, and afterwards with cold alcohol; solution of the washed sediment in warm water; shaking the solution so obtained with pure amylic alcohol, which extracts the uroerythrin with great avidity; evaporation of the amylic solution, and solution of the residue in distilled water.

Such solutions of uroerythrin are, as Riva and Zoja have shown, rapidly decolorised by light, and must therefore be kept in a dark place, or must at least be protected from actinic light.

Uric acid crystals, thrown down from solutions of uroerythrin, have a delicate pink colour, which is quite unlike that of the natural urinary crystals, and is more absolutely pink the purer the material employed. The prevailing shape recalls that of the "razor shell" of our coasts, and the individual crystals are apt to be grouped together into rosettes, or to assume a cruciform arrangement. (Plate III. Fig 2.)

Similar pink crystals may be obtained in a simpler way by allowing the aqueous solution of a deeply-coloured pink urate sediment, which has been thoroughly washed both with water and with alcohol, to stand in a dark place. Rosettes and clusters of prismatic crystals are in time deposited, together with a few boat-shaped ones, which have a yellow tint; for urate sediments always contain some yellow pigment, which is not removed by washing.

It is evident from the above results that uroerythrin has a great affinity for uric acid, and when present in the urine must have a share in the coloration of the crystals deposited therefrom; but it is equally evident that uroerythrin is not the only colouring matter of the urinary sediments. The presence of some uroerythrin in them is the rule rather than the exception, for, as Riva and Zoja have shown, by shaking the urine with amylic alcohol the presence of this pigment may very frequently be demonstrated even in pale urine; and it is well known that the urine of healthy individuals not infrequently deposits pink urates.

A further series of experiments showed that urobilin, which is by some regarded as the chief colouring matter of normal urine, is not one of the pigments concerned in the coloration of the deposits; for it was repeatedly found that uric acid crystals, thrown down from solutions of urobilin, were hardly appreciably tinted when examined in bulk, and under the microscope appeared practically colourless. Moreover, and this is a point of considerable importance, the crystals assumed forms identical with those met with in specimens deposited from pure aqueous solutions of urate.

Hæmatoporphyrin, extracted from urine, proved to be equally devoid of the power either of colouring or of modifying the form of uric acid crystals.

Of the pre-formed urinary pigments there remains to be considered the yellow colouring matter of normal urine, to which Thudichum has assigned the name of Urochrome. Of the existence of such a pigment,

as distinct from urobilin, I am convinced, and in a paper recently communicated to the Royal Society¹ I described in detail a method by which it can be isolated, in a condition of approximate purity, which differs from those previously employed in dispensing with the use both of mineral acids and of metallic precipitants.

Urate sediments always contain some of this pigment, and from the palest urate sediments, which show no pink tint, aqueous solutions may be obtained which deposit "whetstone" crystals of a pale yellow colour. In some cases there are found in the urine—together with such uroerythrin—free urate sediments, crystals of uric acid which have a yellow or brownish-yellow colour when examined under the microscope, and which appear yellow or fawn coloured when seen in bulk. There is, therefore, good reason for supposing that such sediments, which constitute the second group in the classification given above, do not owe any of their colour to uroerythrin.

Numerous experiments were made by dissolving pure colourless urate in solutions of the yellow pigment, prepared by the method above referred to, and it was found that this substance had a very great affinity for uric acid. It was, however, much more difficult to obtain satisfactory specimens than when solutions of uroerythrin were employed.

The uric acid was apt to be deposited very rapidly in the form of an almost impalpable powder, which consisted of very minute and faintly tinted crystals, but these showed a marked tendency to assume the whetstone form. Aqueous solutions of the isolated yellow pigment are very prone to undergo decomposition, which is evidenced by a change of tint from yellow to brown, and this tendency, which is especially pronounced when the liquid is rendered acid, introduces a further difficulty, and when larger and more slowly formed crystals were obtained they usually showed a brownish tint (Plate III. Fig. 3). The most satisfactory specimens bore a very close resemblance, both in colour and form, to the paler variety of urinary sediments; and there cannot, I think, be any doubt that it is this yellow pigment that supplies the ground tint of the natural crystals, and plays a conspicuous part in moulding them to the whetstone shape, or to the more complex forms produced by the agglomeration of the boat-shaped crystals.

Since the yellow pigment yields no spectroscopic absorption bands, and gives no characteristic reactions by which it can be recognised with certainty in such minute quantities as are here available, there is little hope of demonstrating its presence in the crystals by other methods; but it can easily be shown that uroerythrin exists in "cayenne pepper" sand in the following way:—

The washed sediment is boiled in water or alcohol until its colour is to some extent removed, and the coloured extract is evaporated to dryness.

¹ *Proc. Roy. Soc. London*, 1894, vol. lv. p. 394.

The solid pigmentary residue so obtained is then touched with a glass rod, which has been dipped in a solution of sodium or potassium hydrate, when a green colour is developed at the point of contact.

This property of yielding a green colour with alkalies is peculiar to uroerythrin among the urinary pigments, and by means of this test the presence of uroerythrin may be demonstrated in crystals which have only a light red colour in bulk, and individually have a bright orange tint.

Moreover, such crystals may be found embedded in pink urate sediments, and sometime exhibit a distinct pink tint near their edges.

It would seem, then, that the pigments concerned in the coloration of cayenne pepper sand are the yellow pigment and uroerythrin, and by the admixture of these two substances, in varying relative proportions, the majority of the tints observed in the naturally deposited urinary crystals may be explained. On the other hand, those sediments which lack the usual red colour, when seen in bulk, do not contain uroerythrin, but may be supposed to owe their tint to the yellow pigment alone.

This interpretation of the observed phenomena affords no explanation of the presence of iron in the sediments. Like Kunkel I have detected traces of this metal in specimens thrown down by hydrochloric acid, as well as in the natural sediments; indeed, the natural deposits yield, as a rule, a much more conspicuous iron reaction than do those precipitated by acid, and when burnt on a platinum dish sometimes leave an appreciable amount of reddish ash. For example, whereas 0.09 grm. of sediment thrown down from normal urine by means of hydrochloric acid gave, after combustion, only a very feeble colour reaction with potassium sulphocyanide, such much smaller quantities of natural sediments as weighed only 0.0152 and 0.0293 grm. yielded, when similarly treated, a very pronounced colour.

It should be mentioned that the amount of iron varies much in different specimens, and bears no obvious relation to the depth of their coloration.

It can be definitely stated that the iron contained in the crystals is not contained in the yellow pigment or in the uroerythrin, and if, as Kunkel suggests, it is a constituent of the included colouring matter, it must be concluded that some other pigment than those mentioned has a share in the coloration of the crystals, and one very rich in iron, seeing that the sum of the included pigments constitutes but a very small fraction of the total mass of the sediments. Of the presence of such an iron-containing pigment there is no evidence other than this, but the point requires further investigation, since it promises to throw light upon the nature of the unknown compound that contains the small amount of iron, which is constantly met with in urine.

In the yellow pigment, as obtained by my process, I have, indeed, detected extremely minute traces of iron, almost certainly of the nature

of an impurity, for no appreciable reaction with sulphocyanide was obtained after the combustion of specimens of the isolated pigment exceeding in weight the specimens of natural uric acid sediment dealt with in the experiments referred to above.

Specimens of uroerythrin gave, after combustion, no sulphocyanide reaction, and although the quantities employed were small, and did not exceed 4 or 5 mgrms., it may, I think, be confidently stated that uroerythrin is also an iron-free pigment.

Among the abnormal pigments which modify the colour of the sediments of the third group, those of the bile occupy a prominent place.

As the late Professor Ultzmann of Vienna used to point out, sediments of uric acid, deposited in urine containing bile pigments, have a brown instead of a red colour. The tint varies considerably according to the extent to which the conversion of bilirubin into biliverdin has taken place. In urines rich in bilirubin the deposits have a ruddy brown colour, and the tint of the individual crystals is an unusually rich and warm orange; but when, on the other hand, there is much biliverdin present the colour of the deposits is leathery brown, and the individual crystals show a peculiar greenish tinge (Plate III. Fig. 4). The forms of the crystals are also conspicuously modified, especially by biliverdin, and in some instances urines rich in this pigment deposit rosettes of prismatic crystals, whereas in other specimens which I have examined the deviation from the usual urinary types has been less marked.

Blood pigments have no such effect, and sediments deposited from urines containing blood show no modification of tint.

The dark brown or black products formed by the oxidation of phenol derivatives also possess the power of colouring the crystals to a conspicuous degree. My attention was called by Dr. F. W. Andrewes to the fact that in cases of carboluria uric acid sediments, when present are perfectly black. Examined under the microscope the individual crystals are of so deep a brown colour that they are rendered almost opaque (Plate III. Fig. 6).

Lastly, some at least of the little understood brown or black substances which are formed by the action of mineral acids upon urine have a similar power, as is shown by the reddish-brown colour of the crystals thrown down by means of hydrochloric acid (Plate III. Fig. 5).

It is probable that the brown tint of such specimens is largely due to the products of decomposition of the yellow pigment, such as the uromelanine of Thudichum, but it is also possible that the indigo pigments have a share in its production, for from impure solutions of yellow pigment, containing indoxyl sulphate, I have obtained, on the addition of hydrochloric acid, crystals which were obviously tinted with indigo blue. Dr. Ord,¹ too, has obtained crystals encrusted with and penetrated

¹ *Trans. Path. Soc. London*, 1892, vol. xliii. p. 195.

by indigo blue, by the addition of mineral acid to urines rich in indoxyl sulphate.

On the other hand, the indigo pigments cannot be supposed to have any share in the coloration of the naturally deposited crystals, since these pigments are, as far as I am aware, only produced spontaneously in alkaline urines.

CONCLUSIONS.

1. Of the true urinary pigments, which exist ready formed in urine, only the normal yellow pigment (urochrome) and uroerythrin appear to possess the property of colouring uric acid crystals deposited from their solutions.

2. The yellow pigment, being a constant constituent of the urine, always furnishes the ground tint of the crystals, and plays the more important part in determining their form; the whetstone or canoe shape being that which this substance specially tends to produce.

3. In the majority of instances uric acid crystals, which are spontaneously and rapidly deposited from urine, contain uroerythrin also, and it is to this pigment that the sediments owe their red colour when seen in bulk.

4. The various shades of orange and red observed in the individual crystals are due to the admixture, in varying relative proportions, of the above two pigments; and although crystals coloured by the yellow pigment alone are sometimes met with, uroerythrin is never the sole colouring matter of the natural sediments.

5. The minute quantity of iron present in the sediments is not a constituent either of the yellow pigment or of uroerythrin.

6. Other pigments occasionally present in urine, which have a share in the coloration of the crystals in some cases, are the brown products produced by the action of mineral acids, the oxidation products of phenol derivatives, and the pigments of the bile.

7. Urobilin and hæmatoporphyrin take no part in the coloration of the crystals.

[The expenses of this research were amongst those covered by a grant from the Government Grant Committee of the Royal Society.]

DESCRIPTION OF PLATE III.

FIG. 1.—Crystals of pure, colourless uric acid.

FIG. 2.—Crystals of uric acid deposited from a solution of uroerythrin.

FIG. 3.—Crystals of uric acid deposited from a solution of the yellow pigment of urine (urochrome), in which some colourless urate had been dissolved.

FIG. 4.—Crystals of uric acid deposited from a specimen of urine rich in biliverdin.

FIG. 5.—Crystals of uric acid thrown down by the addition of hydrochloric acid to normal urine.

FIG. 6.—Crystals of uric acid deposited from the dark urine of a patient with carbouluria.

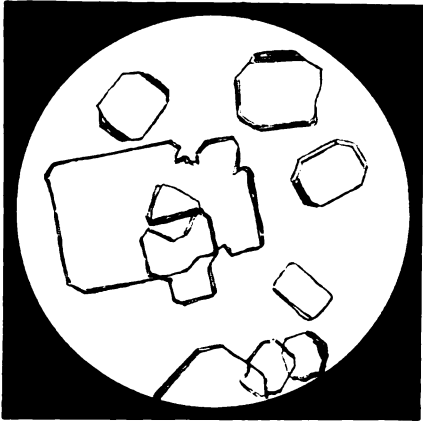


Fig. 1

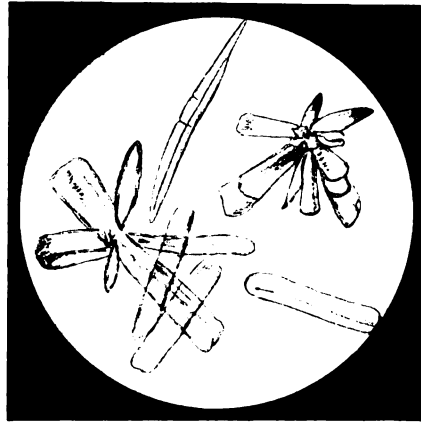


Fig. 2



Fig. 3

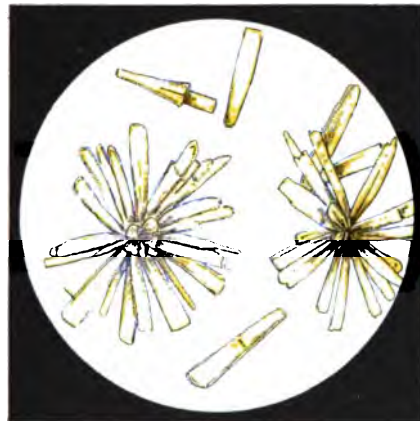


Fig. 4



Fig. 5



Fig. 6

to .vnu
Angela

A NOTE ON THE PERCENTAGE OF IRON IN THE LIVER IN ANKYLOSTOMIASIS.

By BEAVEN RAKE, M.D. (Lond.), *Medical Superintendent of the Trinidad Leper Asylum.*¹

THE researches of Quincke, Peters, Rosenstein, and William Hunter have established the fact that in pernicious anæmia an excessive destruction of red blood corpuscles takes place in the portal circulation. This is shown by an increased percentage of iron in the liver, as compared with other organs.

The presence of the *Ankylostoma duodenale* produces symptoms which resemble so closely those of pernicious anæmia, that a comparison of the pathology of the two diseases at once suggests itself.

Sahli has shown that in some cases the degree of anæmia is not proportionate to the number of ankylostomata present. Reyher and Runeberg have recorded several cases in which there has appeared to be a close connection between an intense form of anæmia and the presence of *Bothriocephalus latus*, without any loss of blood. It has, therefore, been argued by William Hunter and others that in ankylostomiasis the anæmia is not due merely to loss of blood caused by the worms, but to the absorption from the intestine of some poisonous agent, which gives rise to increased blood destruction in the portal circulation.

It has always seemed to me that the drain of blood from the wounds inflicted by the sharp chitinous hooklets of the ankylostoma is fully sufficient to explain the extreme anæmia present in these cases. It is hardly safe to draw conclusions from the number of worms actually present in the intestine at the autopsy, for a purgative medicine or an intercurrent attack of diarrhoea may easily carry off a large quantity of the parasites. Further, the patients who are usually the subjects of ankylostomiasis are not those who would readily endeavour to make good the loss of blood by a generous diet. Many of them are confirmed dirt-eaters.

To examine the question, I obtained pieces of liver from five cases

¹ The MS. of this paper was received only the day before the sad announcement of Dr. Beaven Rake's death was made in this country.—ED.

of ankylostomiasis under my care, and the results of analysis are now recorded. My thanks are due to Mr. P. Carmody, F.I.C., Government Analyst, and Mr. H. Tate, F.I.C., Assistant Government Analyst, for their kindness in estimating the percentages of iron present in the livers.

The following are short notes of the cases :—

CASE 1.—P. H., female, æt. 27. Rapid development of anæmia. Many pints of fluid in chest and abdomen. Heart muscle flabby. Cavities greatly dilated. Small intestine filled with ankylostomata. Liver contains only a trace of iron. Spleen '04 per cent.

CASE 2.—B. H., female, æt. 40. Intense anæmia. Edema of extremities. Somewhat less after taking thymol. Plenty of bright yellow subcutaneous fat. Swarms of *Ankylostomata* in duodenum, fewer in jejunum, very few in ileum. Large quantities of dark, tarry blood throughout the small intestine. At the lower part of the ileum the blood shows black through the intestinal wall. Liver, 20·72; fatty; very anæmic. Ammonium sulphocyanide gives pale red colour, especially at periphery of lobules. Potassium ferrocyanide darkens sections, but no blue colour is produced. Iron, '26 per cent. Spleen, 4·02. Ammonium sulphocyanide gives dark red colour, generally diffused. Potassium ferrocyanide gives pale blue colour; iron, 3·28 per cent.

CASE 3.—J. H., male, æt. 57. Persistent anæmia. Loud systolic bruit, confined to apex. Dyspnœa. Eats earth. Grey hepatisation of right lung. Heart, 9·02; left ventricle hypertrophied. Aortic valves atheromatous. Several ankylostomata in jejunum, and much dark blood and mucus. Liver, 28·02; iron, '2068 per cent. Spleen, 7·02.

CASE 4.—C. H., male, æt. 54. Intense anæmia. Pulse weak. Edema of extremities. Plenty of subcutaneous fat. Grey hepatisation of right lung. Heart, 12·02; hypertrophied. In duodenum are great quantities of ankylostomata, some of them containing blood. Liver, 35·02; iron, '0123 per cent. Spleen, 12·02; hypertrophied; iron, '0592 per cent.

CASE 5.—G. H., male, æt. 56. Anæmia not marked in skin. Pneumonia of right apex. Numerous ankylostomata in duodenum and jejunum. A good deal of dark, tarry blood and bile in intestine. Liver, 32·02; iron, '0228 per cent. Spleen, 8·02; iron, '071 per cent.

The iron in the five cases may be seen at a glance in the following summary :—

Case.	Liver.	Spleen.
1 . . .	Trace	'04
2 . . .	'26	3·28
3 . . .	'2068	Not examined
4 . . .	'0123	'0592
5 . . .	'0228	'071
Average percentage .		<u>'862</u>

In the first analysis the precipitation method was adopted, and it was found that ammonia gave only a slight coloration with the liver ash dissolved in hydrochloric acid. No precipitate could, therefore, be obtained for weighing. In the other four cases the solution was titrated.

In a table given by William Hunter, in his paper, it is shown that in

8 analyses of the liver in cases of pernicious anæmia by Quincke, Zaleski, Rosenstein, and Stahel, the average percentage of iron is '713, the range being from '364 to 2·01. In 14 analyses of the liver in other diseases by the same observers the average percentage is '203.

The spleen was only examined in four of the five cases. Each analysis showed more iron than was present in the liver of the same patient. In one case the large percentage of 3·28 was found. This may have been due to an effort on the part of the spleen to repair the loss from the liver. In pernicious anæmia the percentage of iron in the spleen is, as a rule, but little affected, and, as far as can be inferred from the above figures, the same fact obtains in ankylostomiasis.

From the analyses given above, the following conclusions can be drawn:—

1. In ankylostomiasis the average percentage of iron in the liver is less than in other diseases, and is very much less than the average percentage in pernicious anæmia. Ankylostomiasis = '1 Other diseases = '12. Pernicious anæmia = '7.
2. The iron in the spleen is scarcely affected in ankylostomiasis.
3. The intense anæmia associated with ankylostomiasis is simply due to loss of blood from the intestine, and is not caused by any toxic blood destruction in the liver.

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A CONTRIBUTION TO THE STUDY OF CALCAREOUS CONCRETIONS IN THE BRAIN.

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From the Pathological Anatomical Institute of Professor Chiari in Prague.

(PLATE IV.)

THE case which forms the basis of this paper showed two pathological processes present in a marked degree and intimately combined in the blood vessels of the brain, leading to atrophy of considerable portions of nervous tissue, without there having been noted during life any abnormal mental symptoms.

These two pathological processes are *colloid infiltration* and *calcification*. The calcification only was detected macroscopically, and it was noted as occurring in three forms—as rigid blood vessels projecting like ends of wire from the cut surfaces of the brain, as fine sandlike deposits, and as stonelike concretions up to the size of barley grains.

The case was studied with particular reference to three points, namely, the location of the colloid material with reference to the blood vessels, the relation between the colloid material and the calcification, and the origin of the sandlike deposits and stonelike concretions.

The following is extracted from the *autopsy* record:—

WOMAN, 45 years old.—*Autopsy*, April 29th, 1891. Surgical Clinic, Prof. Gussenbauer.

Clinical diagnosis.—Morbus Brightii chronicus. Parotitis dextra. Bronchitis catarrhalis. Pneumonia lobularis sinistra.

Pathological anatomical diagnosis.—Morbus Brightii chronicus cum atrophía granulari renum. Hypertrophía excentrica ventriculi cordis sinistri. Endarteritis chronica deformans. Parotitis suppurativa dextra. Pneumonia lobularis bilateralis. Calcificatio arteriarum cerebri et cerebelli. Nodi calcarei cerebelli.

Brain (Mus. No. 4745).—The scalp pale. The calvaria 49 cm. in circumference, long, thicker especially in the anterior and posterior parts. The sagittal suture obliterated. The dura more adherent than normal to the internal surface of the skull. The internal meninges not thickened, easily removable from the surface of the brain. The basal arteries in general thin walled. The ventricles of the brain not dilated. The substance of the brain pale, and a little more moist than normal. In the pons numerous fresh punctate hæmorrhages. In the white substance on all the cut surfaces of the whole cerebrum numerous small rigid calcified arteries projecting from the brain substance like ends of wire.

The same alteration of the blood vessels as in the cerebrum on the horizontal cut surfaces through the cerebellar hemispheres in the dentate nucleus and in the medullary substance. Here and there also fine sandlike deposits in the brain substance of the cerebellum; and, finally, in the inner layer of the cortex of the cerebellum stone-like concretions up to the size of barley grains.

The material for *microscopical examination* consisted of a vertical section through the gyrus centralis anterior of the right half of the cerebrum (sectio frontalis of Pitres), of the whole of the right half of the cerebellum, and of the pons and medulla. The position of each piece, cut and mounted from the above mentioned parts, was carefully noted, so that, when the work was finished, and the drawings of the various slide preparations were joined together, they showed one frontal section through the whole of the right half of the cerebrum, and three frontal sections through the right half of the cerebellum, one through, the other two anterior and posterior respectively to the dentate nucleus.

In the pons and medulla no pathological changes in the blood vessels could be found.

The intima of the basilar artery was considerably thickened in places from chronic endarteritis, but the vessel showed no other changes.

The pieces of brain tissue were imbedded in celloidin. After a number of sections had been cut, the blocks were put into 5 per cent. nitric acid for from 1 to 2 days, to decalcify. They were then thoroughly washed in water, and hardened in 80 per cent. alcohol for cutting again. In this way the differences between the sections before and after decalcification could easily be compared. The most useful stain was found to be Delafield's hæmatoxylin. Sections were stained in the strong solution for from 1 to 4 minutes, and then left in plenty of distilled water over night. By this method the colloid material was always sharply defined, taking a considerably deeper stain than the nuclei, and of a slightly blue tint. The calcified material either did not stain, or acquired a somewhat reddish blue tint. The fatty crystalline deposits often present in great abundance (on account of the calcification the tissue had been hardened in alcohol), were but lightly and rather diffusely stained, so that they could easily be distinguished from the colloid. Alum cochineal was also used, but the differentiation of the various parts was not so good. For contrast between the colloid material and the nuclei, Van Gieson's method, or even better, Weigert's fibrin stain was found serviceable.

A number of the stonelike concretions were picked out, decalcified, and sections cut and stained. Numerous arteries and capillaries were also teased out. Some were mounted before, others after decalcification, others again were stained. Tested with strong sulphuric acid, the calcified vessels were quickly covered with innumerable fine needle-shaped crystals.

Before beginning the description of the pathological changes found

microscopically, it may be stated that the lime salts were found nowhere except in transparent colloid material as a basis for the deposit.

Cerebrum.—The changes in the arteries, in the precapillaries and capillaries, and in the veins will be taken up in order.

Nearly all of the *arteries* had calcified walls, often of great density. The middle coat was always the first and most markedly affected (Plate IV. Fig. 1). The process gradually extended, however, into the adventitia, and not infrequently also into the lumen of the vessel, gradually obstructing it. After decalcification a homogeneous, highly refractive, transparent substance was found occupying the position previously held by the lime salts. In the earliest stages of the process in the muscular layer the transparent drops seemed to lie to some extent within the muscle fibres, and even to encroach upon the nucleus. But judging from the way in which the colloid material spread out and coalesced into irregular masses, the change must also take place between the cells. As the process advanced, the muscular coat disappeared. In the adventitia small transparent drops made their appearance, often coalescing into irregular masses. Even in the most calcified arteries, however, a trace of the adventitia could usually be found on the outside of the vessel. On the inside of the arteries the colloid material often projected into the lumen irregularly (Plate IV. Fig. 2); or in the form of a broad or narrow ring, gradually occluding it (Plate IV. Fig. 3). So long as the vessel remained pervious, the endothelial cells of the intima were unaffected.

In the *precapillaries* and *capillaries* the colloid material made its appearance as fine drops in the wall between the inner and outer surfaces, and apparently irregularly, without reference to the nuclei of the cells (Plate IV. Fig. 4). Even the very smallest drops stained intensely with hæmatoxylin. They gradually increased in size and joined one another so as to encase the vessel in a thick, homogeneous, highly-refractive, nodular wall (Plate IV. Fig. 5). Some of the masses in the walls were much larger than others, and had a concentric appearance. Excepting the very small drops, all this colloid material was infiltrated with lime salts.

In the *veins* the colloid material was present in much less abundance, usually as small or large drops in the media, or in the inner layer of the adventitia (Plate IV. Fig. 6).

The changes in the *precapillaries* and *capillaries*, which formed the most interesting feature of this case, were not equally distributed throughout the brain substance of the cerebrum, but were confined mainly to certain localities. They were to be found to some extent in the white matter, more particularly in the neighbourhood of the ganglia, but they were most extensive here and there in those parts of the grey matter of the cortex which were nearest the ganglia, as, for instance, at the base of the sulcus between the middle and inferior frontal convolutions and in the basal ganglia. The largest of these areas measured

over 1 cm. in extent. In these places nearly the whole of the anastomosing network of blood vessels was changed into a calcified, irregular, nodular, branching framework, showing in the spaces occasional ganglion and small cells, with here and there a blood vessel whose walls showed no pathological change. In a few small areas the spaces between the capillaries were filled with calcified colloid material, so that small concretions were formed.

In the *cerebellum* the pathological changes were even more remarkable and interesting than in the cerebrum. All of the arteries lying in the white matter and within the dentate nucleus had calcified, often nodular walls, and not infrequently the lumen was occluded. But it was in the smallest blood vessels that the greatest change had taken place. In the dentate nucleus all the capillaries, almost without exception, running between the ganglion cells had thickened calcified walls, and formed an intricate network, in the interstices of which were a few ganglion and small cells (Plate IV. Fig. 7). In places the tissue between the capillaries had completely disappeared, and the spaces had become filled up with calcified colloid material binding the vessels together, so that concretions were formed.

In the granular layer of the cortex, nearest the central white matter, the capillaries had become transformed in the same manner as in the dentate nucleus, into a calcified network, but the intervening spaces had become filled up to a much greater degree by colloid material which afterwards had become calcified, so that numerous very hard nodular concretions had been formed often measuring 5 mm. in length and 2 to 3 mm. in diameter.

All of the concretions picked out of the brain tissue of the *cerebellum* proved, on examination, to be either portions of the granular layer or of the dentate nucleus. A section through one of these concretions after decalcification showed many wavy, twisting lines, usually arranged in many layers (Plate IV. Fig. 8). The colloid substance in these areas, judging from its reaction to stains, was of varying density. The capillaries could still be recognised by their deeper colour, and it was around them that the various successive layers of colloid had been deposited. This could be seen to best advantage in cross sections of the vessels.

In many places the granular layer had been almost completely destroyed. The molecular layer surrounding these concretions was frequently reduced to a half, or even a third of its normal thickness. Purkinje cells could still be found, but they were very few in number.

The capillaries in the molecular layer afforded the best opportunity for the study of the deposit of the colloid substance, for the process here was rarely far advanced. In a few places, however, the capillaries here were changed into many-layered, solid, contorted tubules, which, on section, gave the appearance of round, oblong, and pear-shaped concentric bodies, little resembling the vessels from which they arose (Plate IV. Fig. 8).

No pathological changes could be found in any of the vessels of the pia mater of the cerebrum or cerebellum.

In connection with this case two other cases of "simple calcification" of the cerebral arteries were also studied.

The first (Mus. No. 2654) was from a man sixty-two years of age, who died from *morbus Brightii* complicated with ascites. There was found marked calcification of many of the arteries. The middle coat was most affected, but there were always numerous small round and irregular masses in the adventitia, and not unfrequently the lumen was occluded. In some of the capillaries were drops of colloid, of which the larger were calcified. After decalcification all the colloid substance stained deeply with hæmatoxylin.

In the second case (Mus. No. 2385), from a man fifty-six years of age, who died from emphysema complicated with ascites, the calcification of the arteries was also very marked, and microscopically the same appearances were found as in the case above. Many of the capillaries contained in their walls drops of colloid which were often calcified.

The study of these three cases shows that the colloid infiltration takes place in the arteries in the brain first, and most extensively in the middle coat, that it is found early in the adventitia but to a much less extent, and that, as a rule, the intima is last invaded as the vessel becomes occluded. It shows also that the deposit of lime salts takes place into the colloid material. The fine sandlike deposits were undoubtedly due to the calcified capillaries, and the stonelike concretions to the masses of capillaries bound together by calcified colloid material.

This manner of formation of concretions throws considerable light on a noted case reported by Bamberger and cited by Rokitsansky¹ in his "Pathological Anatomy."

In the brain of a woman thirty-four years old, for many years insane and subject to epileptic seizures especially at night, there was found besides calcification of the arteries, which "stuck up from the cut surface of the brain like the ends of so many wires," circumscribed areas in the corpora striata filled with yellow stonelike conglomerations.

Speaking of calcification in the brain in general, and of this case in particular, Rokitsansky says: "There occur in the brain in the white substance, and also in various parts of the brain provided with grey matter, especially in the corpus striatum, for instance, areas in which the brain substance is replaced by a considerable conglomeration of simple and layered, smooth or nodular, yellowish, stony masses fragile as glass, which are identical in formation and substance with the sandlike deposits in the pineal gland. They are stuck together like glands of considerable size, and extending through the whole mass is a framework composed of vessels and of bundles of fibrous tissue. In these areas is to be seen a growth of connective tissue caused often, perhaps, by encephalitis, in which the fragments of the nerve tubes are ossified. A considerable

¹ Rokitsansky, "Pathologische Anatomie," bd. ii. p. 472.

number of the incrustations always adhere sheathlike to the vessels. In rare cases the blood vessels of the brain are surrounded by these masses in the shape of solid sheaths, also outside of the above mentioned areas."

I have quoted this description in full, on account of the accurate picture given of the appearances found in teased preparations. In all probability the areas of concretions described were calcified capillaries, such as were found in the first case detailed in this paper. Therefore, in the light of that case, I cannot agree with Rokitsansky in regarding these concretions as the calcified remnants of nerve tubes.

Holschewnikoff¹ reports an interesting case of a woman, 68 years old, in whom no brain symptoms were noted during life. Besides a "papilloma" beneath the cerebellum, the size of a small apple, and a similar tumour within the cerebellum, there were present in many parts of the cerebrum, but especially in the large ganglia, numerous areas of the size of pinheads and a little larger, which not infrequently contained friable, apparently calcareous, masses. Microscopically, after decalcification, sections through these areas showed, peripherally, colloid in beaded form, suggestive of capillary vessels; in the centre were masses and clumps of colloid which did not resemble capillaries, at whose expense they had been formed. In the tissue surrounding these areas was a network of capillaries with colloid masses, or drops in the walls. In the rest of the cerebrum he found veins with colloid in the adventitia, or in all three coats. The arteries were but little changed. This case can certainly be classed with the one that forms the basis of this work. But the process is much less extensive, and is confined to the cerebrum.

A third case worthy of notice is reported by Simon.² In the cerebellum of an idiot, sixty-nine years old, there was found in the right hemisphere an irregularly round diseased area, somewhat over 2 cm. in diameter, sharply limited by normal nervous substance. In the middle of the white matter of the left hemisphere was found a similar but smaller area hardly $\frac{3}{4}$ cm. in diameter. Both areas were softer than the brain substance, but contained hard spots. In teased preparations were found "long, many-branched threads, undoubtedly calcified blood vessels," also round and long calcified bodies up to 3 mm. in length. Nearly the whole capillary system, with the branches of the smaller arteries, was calcified, and appeared everywhere as composed of round calcified masses, which had become more or less melted together. The other form was like the grains of sand in psammomata. Simon concludes that the softening was secondary to the calcification of the vessels.

It is not at all improbable that it was especially the arteries and capillaries of the dentate nucleus that were involved in these diseased

¹ Holschewnikoff, "Ueber Degeneration der Hirngefäße," *Virchow's Archiv*, bd. cxii. s. 552.

² Simon, "Ausgedehnte Verkalkung der Hirngefäße bei einer Idiotin," *Virchow's Archiv*, bd. lv. s. 534.

areas, but without sections of the areas *in situ* no positive statement can be made.

There are other cases of concretions in the brain cited by Virchow, Lindsay, and others, which undoubtedly can justly be classed as owing their origin to the same degenerative processes in the blood vessels as are illustrated by the above mentioned cases; but these other cases are reported so briefly that it is impossible to determine their exact nature from the descriptions given.

For the term *colloid* which I have used in this paper, others would use the term *hyaline*, introduced into pathology by von Recklinghausen. If I still cling to the old name it has been done for the following reason. In the past there has been understood in pathology, under the term colloid, a degeneration of tissue elements into a substance which is homogeneous and not soluble in the fluids of the body. In the group of substances thus distinguished, mucin degeneration and amyloid formation have received separate names, because these forms of colloid, in the broad use of the term, are distinctly characterised by special chemical reactions, which render it possible to differentiate them from other forms of colloid. For these other forms it seems to me best to retain the term colloid, until each of these certainly different substances can be distinguished by some chemical reaction. I can find no reason for employing now a new name for this group, because the chemical reactions for the hyaline described by von Recklinghausen are not sufficiently characteristic. Moreover, it is to be noted that von Recklinghausen includes in his group of *hyaline*, formations which are certainly very different from the colloid substances, namely, the homogeneous masses in the diphtheritic membranes, and in blood coagulations.

The term colloid *infiltration*, instead of *degeneration*, of the blood vessels has been used, because the generally accepted view at present seems to be that the process is more an infiltration than a degenerative process.

Colloid infiltration of the blood vessels is not at all rare, especially in the brain, and it often occurs without calcification. The literature on this subject is abundant. In one case which came under my notice, where there was very extensive colloid infiltration of the blood vessels without any trace of calcification, and where death occurred in consequence of hæmorrhage into the basal ganglia, the colloid infiltration in the muscular coat could easily be studied in all its stages. It appeared as fine drops, which coalesced here and there in groups of muscle fibres, and apparently also between them. In the capillaries was abundant hyaline deposit.

A careful examination of the blood vessels in all parts of the body, in connection with the examination of the brain in such a case, might throw considerable light on the subject, and would prove an interesting field for further investigation.

For a study of the above described concretions in the brain it is



Fig. 1



Fig. 2

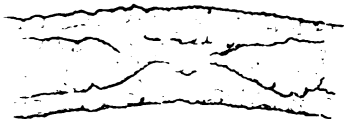


Fig. 3



Fig. 4



Fig. 5

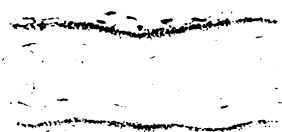


Fig. 6



Fig. 7

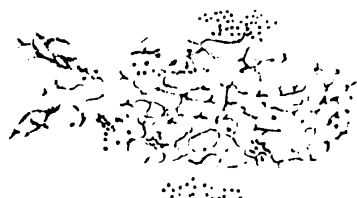


Fig. 8

indispensable, for a proper understanding of them, that they be examined *in situ*, by means of sections through them and the surrounding tissue, as well as in teased preparations.

The following *conclusions* may be drawn from the cases reported and cited:—

Colloid material in the brain is always deposited in the blood vessels. In the larger vessels the middle coat is earliest and most affected.

The colloid material has a tendency to undergo calcification.

The capillary network in certain parts of the brain, especially in the basal ganglia, in the grey matter of the cerebral cortex, in the dentate nucleus, and in the granular layer of the cerebellum, has a tendency, under certain conditions, to undergo colloid infiltration with calcification, leading to atrophy of the included nervous tissue, and to the formation of sandlike deposits, and of stonelike concretions.

I wish here to express my thanks to Prof. Chiari for the material placed at my disposal, and for his kindly oversight of my work.

DESCRIPTION OF PLATE IV.

- FIG. 1.—Calcification of the tunica media in a small artery. (Decalcified). The intima is contracted away from the rigid media by the hardening in alcohol. Zeiss, Obj. E, Oc. 2. ($\times 390$.)
- FIG. 2.—Artery showing irregular calcified mass of colloid projecting into lumen. (Not decalcified.) Zeiss, Obj. AA, Oc. 4. ($\times 90$.)
- FIG. 3.—Artery showing complete occlusion of lumen. (Not decalcified.) Zeiss, Obj. AA, Oc. 4. ($\times 90$.)
- FIG. 4.—Precapillaries and capillaries showing small and large drops of colloid in the walls. (Decalcified.) Zeiss, Obj. E, Oc. 4. ($\times 680$.)
- FIG. 5.—Precapillary showing nodular, colloid, and calcified, sheathlike wall. (Not decalcified.) Zeiss, Obj. E, Oc. 2. ($\times 390$.)
- FIG. 6.—Vein showing colloid drops in the inner half of the adventitia. (Decalcified.) Zeiss, Obj. E, Oc. 2. ($\times 390$.)
- FIG. 7.—Calcified network of the capillary system between the ganglion cells in the dentate nucleus. (Decalcified.) Zeiss, Obj. AA, Oc. 2. ($\times 90$.)
- FIG. 8.—Section through a concretion in the cortex of the cerebellum. The holes are mainly due to portions of the colloid substance falling out of the section. (Decalcified.) Zeiss, Obj. AA, Oc. 2. ($\times 90$.)

OBSERVATIONS ON THE RÔLE OF LEUCOCYTES AND GIANT CELLS IN EPITHELIOMA OF THE TONGUE.

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(PLATES V. TO VII.)

RECENTLY I have had the opportunity of studying 4 epitheliomata of the tongue and 2 of the lip. These tumours have lately excited special interest, since, by the application of the modern methods of fixing, hardening, and staining tissues, a series of structures may be seen which have been interpreted, by several observers, as parasites, interpretations which, though now shown for the most part to be erroneous, are explained, if we look at the special structure of these tumours. There are so many kinds of tissues, and the epithelial cells are seen in such different stages, from that of karyokinesis to that of complete keratinisation, with all the stages between them, that it is sometimes very difficult to succeed in thoroughly differentiating between nucleus and protoplasm. At anyrate sections of these tumours, treated with a double or triple stain, may present such a very intricate picture that one may take a group of leucocytes (when inside an epithelial nest, but standing out from the epithelial cells) for young sporozoa, as happened to Wickham and others. Or, an epithelial cell in an advanced stage of keratinisation, the protoplasm of which has been stained by the safranin or methyl-green nuclear stain—just like a certain stratum of the normal epidermis—may be interpreted as a new parasite, the *Rhopalocephalus carcinomatosus* of Korotneff.

I should like, here, to call attention to a series of phenomena which, though certainly not overlooked by former observers, may receive an entirely different interpretation, and assume a greater importance, now that bacteriologists have so greatly modified and enlarged our ideas on inflammation. Before entering into any further details, I will say a word on the method of examination adopted.

Most of my specimens were already fixed when I got them, and I gladly take this opportunity of thanking Mr. Plimmer, to whom I am indebted for them. Fixation had been carried on by placing them in

corrosive sublimate solution for 24 hours; if this course had not been followed, the specimens were not so suitable for my work. I then washed out the sublimate with running water for a night, after which the pieces were successively put into alcohol of 30, 60, and 90 per cent., and finally into absolute alcohol, for 24 hours. After having removed the alcohol by means of chloroform and ether, the pieces were embedded in paraffin, in the usual way.

I made all my researches with serial sections. This method, borrowed from the embryologist, cannot be too highly recommended for examination of tumours. The possibility which it affords of examining a given spot in all three dimensions was of the greatest help in my study. The paraffin-embedding method enabled me to make with ease sections $10\ \mu$ in thickness, in ribbons of 8–10 sections. These ribbons were fixed on the slides with Mayer's albumen. The paraffin was then removed with xylol, and all traces of sublimate were got rid of by exposing the slides to the action of tincture of iodine for 15 minutes. After the iodine had been carefully removed by alcohol, the sections were ready for staining.

In most cases I used one of the three following methods of staining:—

1. Mayer's hæmalum (hæmateinalum) as the nuclear stain, with cochineal as a contrast stain. I prefer the hæmatein stain to any of the other hæmatoxylin stains, as its action is more constant and equal.

2. Biondi's triple stain; in connection with which it must be noted that the nuclear (methyl-green) stain is easily removed by the alcohol during the dehydrating process—for the same reason clove oil must be entirely avoided. To prevent this, I put the sections for 12–18 hours in a very weak hæmalum solution (the ordinary solution diluted with 30–40 times its volume of distilled water). After having passed through this bath, the sections are only very faintly stained—the blue hæmatein stain being so weak that by itself it would not be of the slightest use. But if these sections are afterwards stained in the usual way with Biondi's mixture the green nuclear stain is no longer removed during the hydrating process, either by alcohol or by clove oil—the blue hæmatein stain is hidden by the methyl-green stain.

3. I tried Foà's safranin-hæmatoxylin mixture, but with as little success as Ruffer and Plimmer, at any rate in the periods of staining indicated by Foà. I obtained, however, a beautiful safranin stain by the following method (which is a slight modification of the well-known method of Rabl). The sections are placed for 12–18 hours in the above quoted diluted hæmateinalum (1 : 30–40), then half an hour in Foà's mixture or in Babes' anilin-oil safranin solution, or in both. The sections after being kept for 5 minutes in a concentrated watery solution of orange are dehydrated with a concentrated alcoholic solution of orange, and then passed through clove oil and xylol. Sections so treated, if not dehydrated for too long a time, show the nucleoli red, the nuclear network

blue, both standing out very sharply, the protoplasm yellow, and the keratinised epithelial cells purple-red. If carcinoma of the breast be stained in this way, the bodies now interpreted as parasites are very different from the epithelial cell protoplasm, their protoplasm being very faintly yellow, the nuclei a more intense yellow.

In all the epitheliomata of the tongue that I have had an opportunity of studying, I could observe that the development of the cell-nest is often accompanied and followed by an invasion of leucocytes. In some cases this phenomenon was only slightly marked, in others there was a large number of leucocytes surrounding and penetrating the cell-nest. Usually they were more abundant, the nearer the spot was to the surface, and therefore the older it was. Where there was rapid growth of the tumour, and where the epithelial nests were only just indicated, there was scarcely any leucocytosis. It occurred to me, then, to trace the history of this process by combining the different stages into a whole. The drawings may serve to make the following lines clearer, but they are but a faint reflex of what I saw in my preparations. The combination of all stages seems to me to present the following phenomena. Leucocytes are seen first at the periphery of the cell-nests; then they seem to enter the interior of the nest. The epithelial cells at this latter place disappear, the following stages being easily observed. A number of leucocytes, 3-5 or more, seem to be included in a shell. In many cases there can still be recognised in this mantle the remains of an epithelial cell, the protoplasm of which has disappeared. Leucocytes have taken its place. This substitution may, I think, be interpreted as a resorption of the epithelial cells by leucocytes. Usually it is the central cell which first undergoes this fate. In other pictures, which may be interpreted as representing a subsequent stage, the greater part of a cell-nest appears to have been taken away; half, or even the whole of the cell-nest having been substituted or, let us say, resorbed by leucocytes. In some cases the outlines of the disappearing cell-nest may be easily traced, many of the peripheric epithelial cells still showing the characteristics of this formation. In other cases the only traces of the resorbed cell-nest are to be seen in the shape of some connective tissue fibres which run circularly around a heap of leucocytes.

I may here discuss the manner in which leucocytes may possibly penetrate to the interior of the cell-nests. I have already pointed out that leucocytes appear first in the periphery of the cell-nests, and are afterwards found in the centre. It is, therefore, justifiable to suppose that a penetration takes place from the periphery to the centre. Another possible way, indicated in Plate VII. Fig. 11, may also be mentioned. Here we have, in longitudinal section, what in a transverse section may appear as a cell-nest. There is an epithelial plug, the interior of which is filled with a broad strip of leucocytes. Following this band towards the left in the preparation, a part is reached made up of connective tissue and vessels, and it is from this that the leucocytes

grew into the epithelial plug by resorbing its innermost part. If it be conceived that this plug is cut in a transverse direction, an appearance somewhat like Plate I. Fig. 2 is observed.

I have observed that whole cell-nests disappear by the action of leucocytes alone. But, as far as my observation goes, this does not seem to be the common process, another histological element—the giant cell—usually coming into action. A cell-nest may be seen with only one giant cell at the periphery, in another there may be two or three at work, either in the periphery or more towards the centre of the cell-nest, in a third four or five and more, and so on, until finally a whole cell-nest, more or less recognisable as such by its contour, may be almost replaced by giant cells (see Plate VI. Figs. 5–8, which show several steps of the process above described).

What, then, is the function of the giant cells? It does not seem improbable that it is the same as that of the leucocytes. They resorb the epithelial cells. Besides this function, however, my preparations (appearances reproduced in some of the drawings) prove pretty clearly that they are also engaged in the resorption of leucocytes. Thus must I interpret the very common fact that leucocytes are found in the protoplasm of giant cells. When I saw epithelial cells filled with them, I looked upon the leucocytes as the resorbing element, but here I observe, in a further stage, that they in turn are liable to the same fate—intra-cellular resorption. Giant cells are resorbing them. That the giant cell is here the active, the leucocyte the passive, element, may be inferred from the fact that one may find in the same giant cell, besides two or three leucocytes, fragments of epithelial cells, the remains of indistinct half-dissolved tissue, especially the remains of keratinised epithelial cells.

This point requires special consideration before observations on the giant cells are further proceeded with. One stratum of the epidermis, corresponding to a certain degree of keratinisation, retains the nuclear stain; it is blue when hæmatein is used, and red with safranin. Now, one sees perfectly analogous phenomena in the epitheliomata of the tongue. Here are cell-nests, the innermost part of which is stained purple. Sometimes the central cell only, or, it may be, several concentric squamous cells of the plug assume this colour (cell-nest). This staining reaction indicates that the epithelial cells still follow the destiny of normal epidermis, that is, the tendency to keratinisation. It is evident that this phenomenon is only to be seen in slowly growing tumours. Now, if such a keratinised cell-nest undergoes resorption by leucocytes and giant cells, the remains of the epithelial cells still have the above mentioned property of retaining the basic (nuclear) stain. Hence the giant cells in Plate VII. Fig. 11 show a number of such red-stained fragments in their interior (indicated in this and in Plate VI. Fig. 8 by cross-hatching). There can be little doubt as to the meaning of this observation—giant cells have swallowed these epithelial cell-remains and are in the act of digesting them. And if in the same giant cell along with these

particles one finds leucocytes, I should be inclined to think that here, too, the giant cells have a digesting function—the function of resorbing the leucocytes which have done their duty and are now to be removed.

The fact that giant cells show, in the interior of this protoplasm, epithelial cells or remains of such, and, at the same time, leucocytes, is so striking, so constant, and so easily demonstrable, that we wonder it has hitherto escaped general notice. That red blood corpuscles are subject to intracellular resorption is now a well-known physiological fact; new, in my opinion, is the observation of the intracellular resorption of epithelial cells and leucocytes by giant cells.

To recapitulate, I think it is permissible to argue that the process of the digestion of the epithelial cells is always begun by leucocytes, and it is only afterwards that both epithelial cells and leucocytes are resorbed by giant cells. The phenomenon, thus interpreted, is the repetition of what we are accustomed to call chronic inflammation. I may here be allowed to point out especially the analogy with tuberculosis. Experimental study shows that the struggle against the microbe in this disease is begun by leucocytes, polynuclear and mononuclear, but in the later stages the giant cells enter into the process, and take part in the digestion of the dead tissue. I would not, however, insist too much upon this analogy, as the epithelial cell is certainly not a full analogue to the bacillus in tubercle, and of course it is possible that, at some future time, the process of pathological growth of epithelial cells may find a similar interpretation to that of leucocytes in bacillar diseases—namely, it may present a defensive process on the part of the organism, a protective move against the attacks of an enemy with which we are not yet sufficiently acquainted. Starting from the principal normal function of epithelium in epidermis, which is protection against injuries by the formation of a very insoluble substance—keratin—one ought to interpret the proliferation of epithelium as an attempt to encapsule a parasitic enemy. The whole process above described by me would then be that of a digestion and removal of dead or dying tissue. Until fuller evidence of the parasitic origin of epithelioma is forthcoming, that interpretation will remain doubtful as applied to the destruction of epithelial cells by leucocytes and giant cells. But as applied to the digestion of leucocytes by giant cells, it must be the right one. And I see, therefore, in epithelioma of the tongue, a function of giant cells, similar to that which Dr. Woodhead has brought to my notice, connected with the tubercle giant-cell-digestion of degenerated or dying tissue.

In conclusion, I may add a few words as to the origin of these giant cells. Dr. Ruffer has on several occasions indicated to me the possibility that these giant cells may be derived from muscle cells, that we might, perhaps, have something like transition from one to the other; and Plate VII. Fig 10, which was made at his desire, is an attempt to show such a transition. I must confess, however, that I could not find sufficient evidence to justify me in accepting such an opinion. Such a

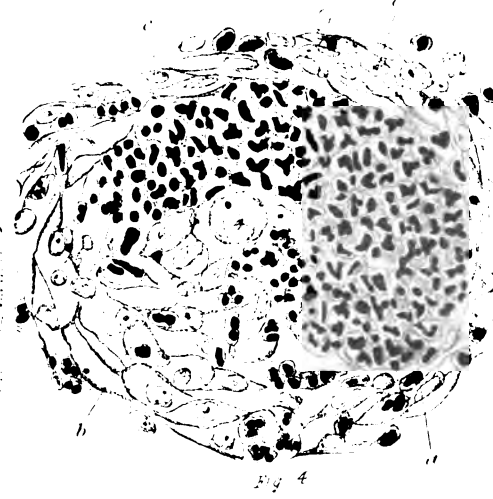
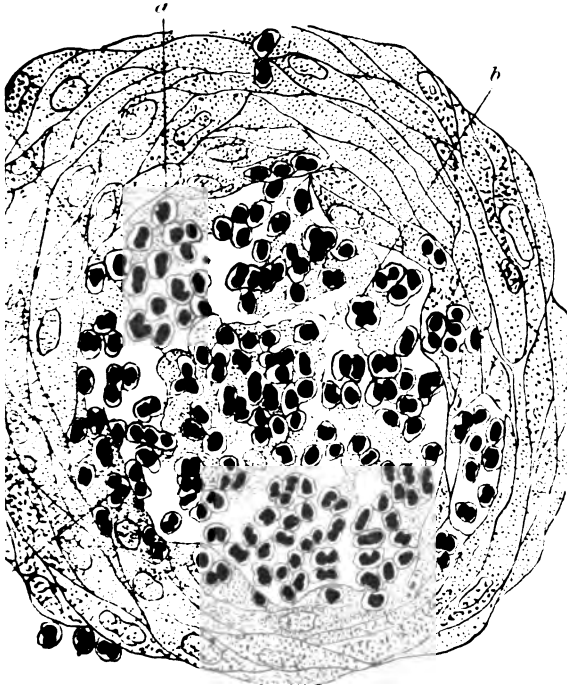
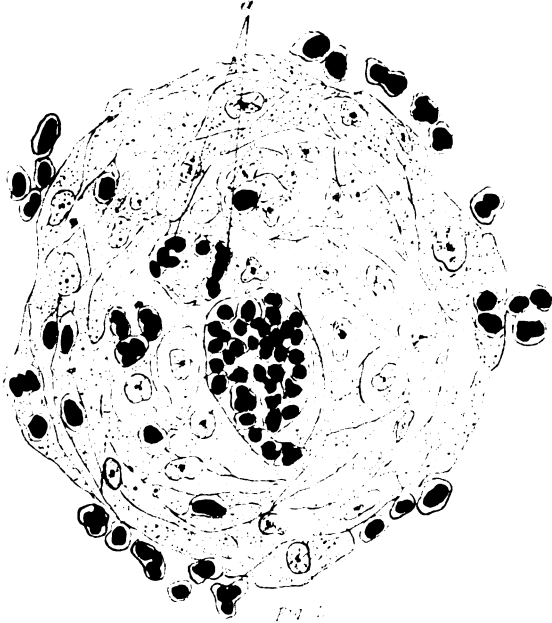
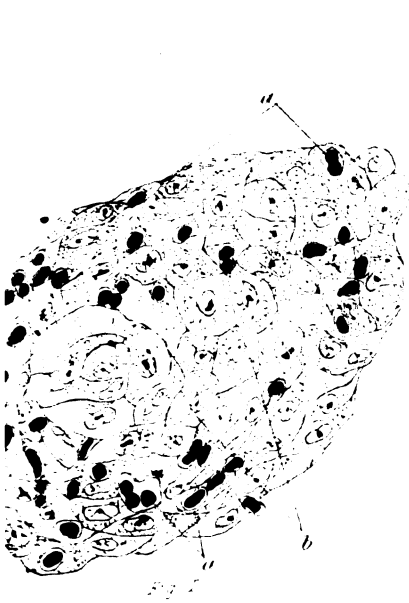
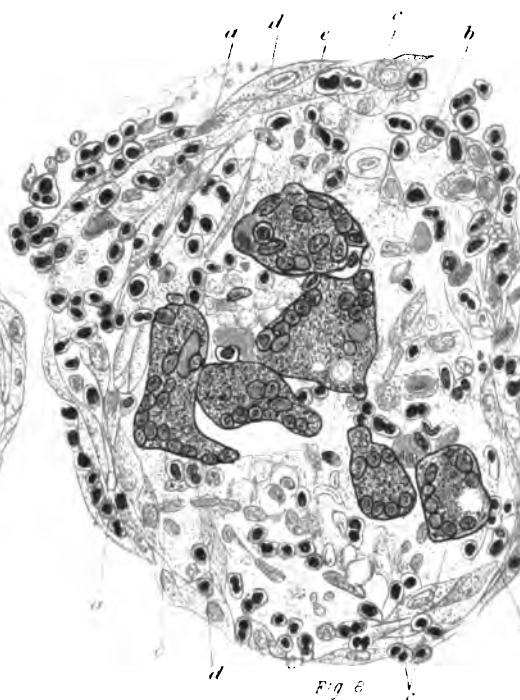
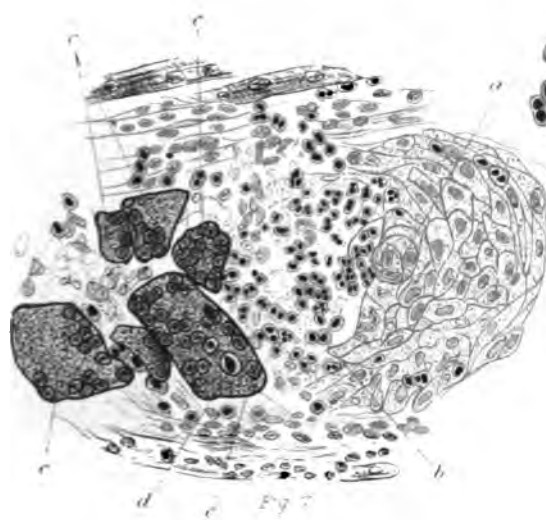
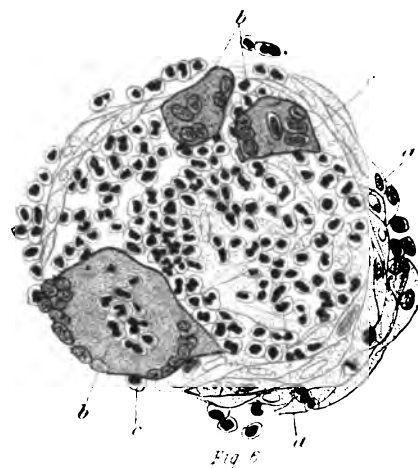


Fig 3

Fig 4

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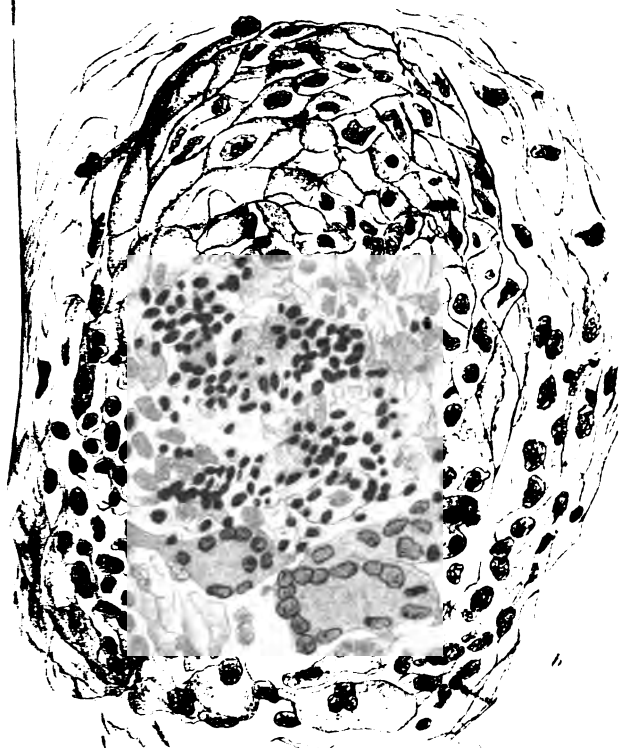


Fig. 1



Fig. 2

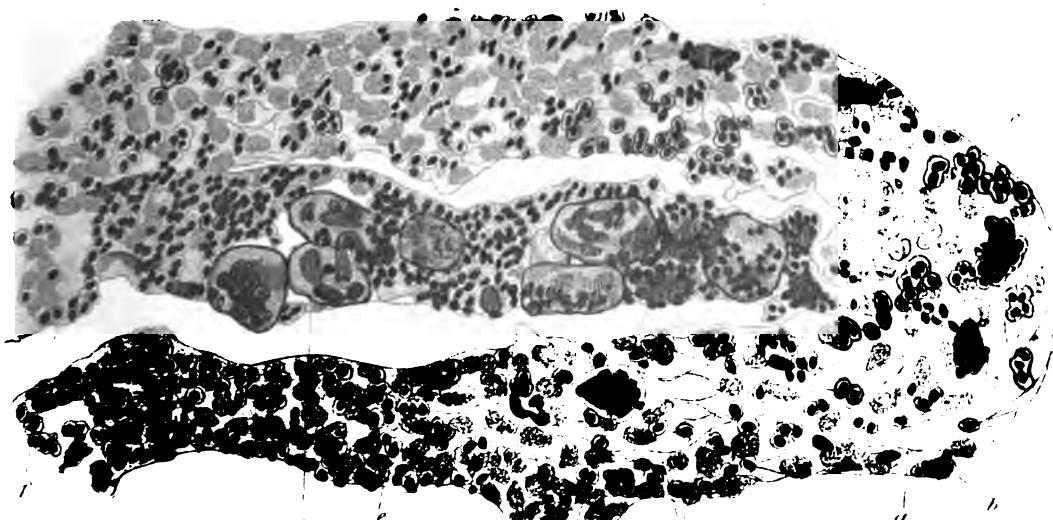


Fig. 3

theory is in opposition to all that we are taught by embryology, which sharply distinguishes striated muscles from any derivative of connective tissue. And, further, no convincing proof of such transition is possible. One may see such a so-called transition cell with still some striations; but then there is no conclusive evidence of the formation of a distinct giant cell. A typical giant cell may be clearly recognised; but, in that case, it has not the slightest relation to a muscular fibre. I, at least, have certainly been unable to show, or to see, any such transition that would afford any satisfactory or convincing evidence of such a transformation.

I take this opportunity of sincerely thanking Dr. Ruffer, who kindly supplied me with the material for this investigation, and helped and advised me repeatedly during this study. I also feel exceedingly indebted to the amiability of Mrs. Ruffer for the beautiful drawings which form the last of my plates. My thanks are also due to Dr. Woodhead, who not only sacrificed his time to control the results of my researches, but with his usual kindness was of the greatest help during the writing of my paper.

DESCRIPTION OF PLATES V. TO VII.

- FIG. 1.—Early stage of penetration of leucocytes. *a*. Into a cell-nest. *b*. Epithelial cells.
- FIG. 2.—Later stage of this process. *a*. Leucocytes resorbing epithelial cells. *b*. Leucocytes which have resorbed the epithelial protoplasm, in a shell of epithelial cell envelopes.
- FIG. 3.—The whole centre of a cell-nest resorbed by leucocytes (*a*). One sees fragments of the resorbed epithelial cells in somewhat shapeless bodies (*b*).
- FIG. 4.—Another kind of resorption of cell-nest. A sector (*b*) of it is still intact, the greater part being replaced by the leucocytes (*a*). Peripheric epithelial cells (*c*) showing the outlines of the whole cell-nest.
- FIG. 5.—Three giant cells approaching a cell-nest (*b*), a part of which (*c*) has been resorbed by leucocytes. In the interior of the giant cells (*a*) are some leucocytes (*d*) in the act of being resorbed.
- FIG. 6.—Innermost part of a cell-nest being resorbed by leucocytes (*a*), three giant cells (*b*), with some leucocytes in their protoplasm (*c*), are resorbing both leucocytes and epithelial cells.
- FIG. 7.—Half a cell-nest (*a*) is still intact, its other half being resorbed by leucocytes (*b*) and giant cells. Leucocytes (*d*) in the protoplasm of the latter.
- FIG. 8.—The outlines (*a*) of a cell-nest are still to be seen, all the rest having been resorbed by leucocytes (*b*) and giant cells (*c*). The bodies (*d*) indicated by hatching are, in the preparation, stained purple by safranin, and signify remains of keratinised epithelial cells. *e*. Leucocytes in the interior of giant cells.
- FIG. 9.—This sketch shows the following section of a series to Fig. 7. *a*. Giant cells. *b*. Leucocytes in their interior.
- FIG. 10.—So-called transition cells from muscles to giant cells (see p. 122 of the text).
- FIG. 11.—Epithelial plug cut in a longitudinal direction. *a*. Epithelial cells. *b*. Keratinised epithelial cells. *c*. Leucocytes. *d*. Broad mass of leucocytes resorbing the interior of the plug. *e*. Giant cells between them. *f*. Transition to a vascular part of connective tissue.

EPITHELIAL CHANGES PRODUCED BY IRRITATION.¹

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(PLATES VIII. TO X.)

RECENT investigations into the minute anatomy of cancerous growths have proved to demonstration our ignorance of the changes to which epithelial cells are liable as a result of irritation. Appearances which may easily be proved to be due to simple irritation have been taken repeatedly to represent protozoa or parasitic growths in cells. Fantastic theories about the origin of cancer have been built upon these erroneous foundations, and the more ignorant the observer, the more he has insisted upon the correctness of his interpretation, until the truth of Goethe's maxim is brought home to us with increased emphasis that "Es ist nichts schrecklicher als eine thätige Unwissenheit"²

The present theory that cancer is associated causally with a protozoon, only a portion of whose life cycle is spent within the cells, has done much good whether or no it ultimately be proved correct. It has led us to examine epithelial cells with the greatest care and by the most exact methods. An insight has been gained by these means into some of the changes which take place in an epithelial cell in the course of its existence, so that what was a short time since only known to one or two of the most expert histologists in Europe is now rapidly becoming a matter of common knowledge to every teacher of the microscopic art.

The investigations leading to the results with which the present paper deals were commenced with the idea of preparing a suitable nidus for the hypothetical protozoon of cancer. In pursuit of this object it became necessary to examine the cells in a large number of irritated epithelial surfaces, to ascertain what appearances were to be attributed to simple irritation, and what changes, if any, took place after the introduction of cancer.³ My experiments last year were chiefly carried

¹ Read in the Section of Physiology at the Oxford Meeting of the British Association.

² "Max. u. Reflexionen," bd. iii.

³ For further details see *Brit. Med. Journ.* 1893, vol. ii. p. 830, and 1894, vol. ii. p. 636.

out upon the vaginal mucous membranes of rabbits and of rats; this year some of the experiments have been repeated, the conjunctiva being substituted for the vagina, as it was necessary to discover whether the effects produced by irritation varied with the situation of the epithelium. The effects of irritating the vaginal epithelium may be summarised briefly as:—A general vacuolation of cells; various forms of œdema; cell nests or epithelial pearls; collections of leucocytes; and the spaces left after these leucocytes have migrated. These spaces must be distinguished from the somewhat similar appearances, with which I shall deal presently, produced by the disintegration and removal of individual cells.

I may say at once that in no case has it yet been possible to produce, by artificial irritation, that remarkable body which has been described with so much care by Dr. Ruffer, the distinguished alumnus of our university, who at present holds the responsible position of Director of the British Institute of Preventive Medicine. There are some bodies which resemble it superficially, but even those which do so most closely are deficient in the clean-cut and perfectly circular outline, as well as in the radial striation which is so distinguishing a feature in the true "cancer body." When portions of cancerous tissues have been introduced to the irritated epithelial surfaces it has twice happened that appearances presented themselves which seemed to be identical with the bodies described by Ruffer, and to be quite different from those resulting from simple irritation. It was possible that these appearances might be due to the introduction of epithelium to the irritated vaginal mucous membrane. Control experiments were, therefore, made upon two rabbits, the piece of cancer being replaced by a fresh cornea taken from another rabbit. Many interesting appearances were obtained, but no "cancer bodies" were observed. It appears, therefore, as if these bodies really bore some relation to cancer, although it has still to be shown that they are not merely some form of modified cell growth.

It is well known that the eye has already been used as a medium for the experimental inoculation of carcinoma, for Leber says:¹ "After the introduction of a portion of a glioma from the eye of a child into the vitreous of the eye of a rabbit the fragment gradually shrank without exciting any inflammation, and led to detachment of the retina." Similar experiments carried out by Messrs. Ballance and Shattock are detailed in the Morton Lecture on Cancer for 1894. These observers introduced fragments of sterile cancer into the anterior chamber of the eye of a rabbit; no change took place in the eye, but the fragment shrivelled. My own experiments are still in progress, so that I need not here dwell upon them; but it occurred to me that the grafting might be more successful if the eye were first brought into a state of chronic irritation. An endeavour was therefore made to procure a suitable amount of irritation by means which would prevent the introduction of

¹ "Die Entstehung der Entzündung," 1891, p. 231.

any fallacy in regard to the subsequent appearances. In one series of experiments, the operation of paracentesis corneæ was performed, on 18th January 1894, upon a healthy guinea-pig and a piece of the bulbous end of a vibrissa was pushed into its anterior chamber. The wound healed by first intention; for three days the cornea was cloudy, and there was a slight circumcorneal zone of inflammation. On 25th January the cornea was clear, the eye appeared to be perfectly normal, and the vibrissa could be seen lying upon the anterior surface of the iris. The cornea was hazy on 3rd February, and two small blood vessels ran into it from the conjunctiva. The animal was killed on the 5th of February; its eye was at once excised and put into Foa's solution. The hardening was completed in 30, 50, 70 per cent. spirit and absolute alcohol. Sections were made by the paraffin method. Photographs of some of the appearances observed in the conjunctival epithelium of the guinea-pig, and in the vaginal epithelium of the rabbit, into whose vagina the cornea was grafted, are reproduced in Plates VIII., IX., and X.

Plate VIII. Fig. 1 shows a cell presenting a more deeply staining central part surrounded by a clear zone. The cell substance outside the clear zone presents obvious radiations, apparently due to some portions of the protoplasm being more resistant than the rest.

Another form of this change is shown in Plate VIII. Fig. 2, where the central and presumably the degenerating portion of the cell is sharply defined, and lies in a clear space resembling a vacuole. The nucleus and the body of the cell remains in a healthy condition.

A similar but still more advanced stage is represented in Plate VIII. Fig. 3, where the cell degeneration appears to have assumed a "colloidal" form, which does not stain readily with hæmatoxylin.

Plate VIII. Fig. 4 shows a similar condition, differing from the previous specimens in the fact that the vacuole contains some solid and more readily staining body, perhaps a piece of chromatin, or possibly the whole body is a swollen leucocyte, so that the preparation might be made to lend support to the theory advanced by Metchnikoff,¹ that such vacuolation in a cell is due to an abundant secretion of digestive juices, and that it is analogous to the vacuolation observed in Protozoa while intracellular digestion is going on.

Plate VIII. Fig. 5 shows a similar state of vacuolation, save that here the degeneration has taken the form of a central mass surrounded by a layer of granules. In Plate VIII. Fig. 6 the granules have fused to form a circumferential layer. The process appears to be complete in Plate IX. Fig. 7, but two cells are involved in the destructive change.

Plate IX. Fig. 8 commences another series of degenerative changes, taking place in epithelial cells as a result of chronic irritation. The cell protoplasm in this case has undergone necrosis, instead of becoming converted into "colloidal" substances.

¹ "Lectures on the Comparative Pathology of Inflammation," translated by F. H. and E. H. Starling, M.D. London, 1893.

Plate IX. Fig. 9 shows that the necrosis which commenced in the neighbourhood of the nucleus has extended towards the periphery, though the whole cell is not yet affected. It is worthy of note, perhaps, that this change is most frequent near the large granular cells, staining red with Ehrlich's hæmatoxylin, which so closely resemble the formative cells found in the segmentation cavity and in the yolk of an impregnated fowl's egg. These cells are probably eosinophile leucocytes. They are extremely numerous in the irritated conjunctival epithelium of the rabbit and guinea-pig. They are found only in the epithelial layers, however, so far as my observations extend at present, and never in the subjacent corium.

The cells are farther degenerated in Plate IX. Fig. 10, though they are not yet involved in irretrievable ruin, as the nucleus and a layer of healthy protoplasm still surround the granular mass.

Plate IX. Fig. 11 shows that epithelial cells of the squamous type possess the power of ingesting other cells. One of the most superficial cells of the conjunctival epithelium has engulfed one of the smaller red blood corpuscles. The microcyte maintains its circular shape, and is enclosed in a vacuole.

This process is still more easily seen in Plate IX. Fig. 12, where there is an amœboid body enclosed in a well-defined vacuole, within a cell of the conjunctival epithelium of a guinea-pig, in whose anterior chamber a vibrissa lay from 18th January to 5th February 1894. The enclosed body appears to have a slightly yellowish tinge, due perhaps to hæmoglobin, but it is, I believe, a leucocyte which has gained admission to the cell. It does not correspond to any of the forms of paranucleus or *nebenkern*, described by Gaule, Platner, Heidenhain, or other observers, but it is exactly similar to an appearance represented by Professor Pawlowsky,¹ and described by him as a parasite, since it was seen in a malignant growth.

A double inclusion has taken place in Plate X. Fig. 13, which is thus a more complex example of epithelial ingestion. It represents a cell containing another cell within it, lying in a vacuole. The including cell itself has a microcyte in its substance, also enclosed in a vacuole. The included as well as the including cells are undergoing degenerative changes.

Plate X. Fig. 14 is another good instance of cell inclusion occurring in surface epithelium. The including cell contains a large vacuole, and within it is a leucocyte. A farther stage of the same process appears to be represented in Plate X. Fig. 15, where two leucocytes are enclosed in the space left by the degeneration of one or more epithelial cells whose horny remains bound the space with a jagged outline. This appearance is easily distinguishable from the somewhat similar space seen in the *Brit. Med. Journ.* 1893, vol. ii. p. 832, fig. 9, where the outline is smooth and the leucocytes are much more numerous.

Plate X. Figs. 16 and 17 belong to another group of cases, for they

¹ *Virchow's Archiv*, 1893, bd. cxxxi. plate xiii. fig. 28.

represent appearances seen after the introduction of scirrhus into the irritated vagina of a rabbit. They are probably only the results of cell degeneration, but the remarkably clean-cut outline of the cell in Plate X. Fig. 17 seems to bring it somewhat nearer to the "cancer bodies." It resembles the appearance drawn by Professor Pawlowsky.¹

Plate X. Fig. 18 shows the appearance met with in the epithelium of a rabbit's vagina a few days after the introduction of a piece of an epithelioma. This figure is an enlargement of fig. 10, published by me in the *Brit. Med. Journ.* last year. The negative is absolutely untouched, but the enlargement has brought into view the radial striation which was before invisible, so that the body still more closely resembles those described by Dr. Ruffer.

I have already called attention elsewhere² to the interesting points of correspondence between Malaria and Carcinoma from a theoretical standpoint. The monographs on Malaria by Marchiafava and Bignami and by Mannaberg have recently been translated into English, and published by the New Sydenham Society, and in this volume will be found a remarkable confirmation of the views I have set out. A comparison of plate i. figs. 28, 29, and 30 in Marchiafava's work and plate ii. figs. 6-10 in Mannaberg's monograph, will show how closely some forms of the malarial parasite approximate the appearances observed by Dr. Ruffer in cancer, and by myself after grafting cancer upon irritated epithelium. The appearances are so similar in the two classes of cases that Marchiafava's description of the parasite in the "summer-autumn tertian" fever applies equally well to the intracellular conditions met with after grafting carcinoma. He says (p. 57): "The phase of the young forms is represented by hyaline plasmodia, without pigment, diaphanous, generally rather large, in size from a fifth to a fourth of that of a red blood corpuscle; there may also be found, along with these, amœbæ of very small dimensions, not larger than a third of the former. These forms, which, as in the quotidian, may be annular and discoid in shape, or display lively movements, are contained, for the most part, in red blood corpuscles of normal aspect." Read epithelial cells for red blood corpuscles, and the description is accurately adapted to the appearances which I have already described,³ and which are reproduced in Plate X. Fig. 18, where it will be seen that the "cancer bodies" vary in size, and that, though two are intracellular, the third lies between the cells as if it had been derived from some extraneous source.

The interest of the present observations lies in the explanation they afford of analogous forms frequently seen in malignant tumours. It is obvious that if similar appearances are not unusual in normal tissues, or in tissues which have only been subjected to slight irritation, they cannot be considered as parasitic when they are met with in cancer or sarcoma. We must become expert histologists before any decided advance can

¹ *Op. cit.* plate xiii. fig. 18.

² *Brit. Med. Journ.* 1894, vol. ii. p. 636.

³ *Ibid.* 1893, vol. ii. pp. 833, 834.

made in our knowledge as to whether cancer is due to a micro-organism or not, for we must be perfectly familiar with the various appearances met with in epithelium which is either normal or is only slightly removed from a healthy condition. The readiest method of doing this seems to be by a prolonged and careful study of epithelial surfaces which have been brought into the condition in which cancer is known to occur clinically; that is to say, with decadent cells and in a state of chronic irritation. When this has been done and done thoroughly we shall be at liberty to look about and to ascertain whether there are any appearances peculiar to cancer, which are not the result of simple irritation. We shall be on the high road to a successful termination of our quest as soon as these are found. Ruffer's bodies at present appear to be instances of such a difference, because they have not yet been found as a result of chronic irritation, though they occur when cancerous growths are brought into contact with irritated cells. We ought, therefore, to use our best endeavours to work out the life-history of these bodies. If they fail us, and prove after all to be only modified cells, we should turn without feeling disheartened to the next most likely form, for it is only by a process of exclusion that we can arrive at the truth. The task appears to be well-nigh endless, for it seems as if the epithelia of different animals had markedly different properties; thus in rabbits degenerative changes are common after irritation, whilst in guinea-pigs cell inclusions seem to be the more frequent result.

I have refrained in this paper from advancing any theories as to the appearances observed, and have contented myself with recording what seem to be facts. The question of phagocytosis by epithelial cells is an exceedingly tempting one to discuss, but at the present time our knowledge of the origin of cancer is more likely to be promoted by careful and correct observation than by adducing new theories. The excellent critical digest by Professor Ströbe, which appeared in Ziegler's *Centralbl. f. allg. Path. u. path. Anat.*, at the beginning of the year, gives no less than 112 references to the work of various observers published from 1890–1893, and even this list is not exhaustive. All these papers are upon the sporozoa causation of cancer. It is therefore of the utmost importance that the foundations upon which such a superstructure has been raised should be examined with the utmost care, lest any flaw therein should lead to its complete destruction.

The explanations I have felt entitled to offer are merely tentative. Few things are more difficult than to describe accurately minute histological changes, and even to determine whether a body is in a cell, on a cell, or below a cell. I have endeavoured, however, by means of photographs to portray the cells as they appear to me at the present time, for there is always in my mind that saying of John Hunter, our great master in the art of observation, "Never ask me what I have said or what I have written; but if you will ask me what my present opinions are, I will tell you."

DESCRIPTION OF PLATES VIII. to X.

- FIGS. 1, 2.—Conjunctival epithelium of guinea-pig. Vibrissa in anterior chamber, January 18th to February 5th, 1894. Foa and Ehrlich's hæmatoxylin. ($\times 600$.)
- FIGS. 3-7.—Vaginal epithelium of old doe white rabbit, iodined September 22nd to November 7th, 1893. Cornea of healthy rabbit in vagina, November 7th to 10th, 1893. Foa. Ehrlich's hæmatoxylin. ($\times 600$.)
- FIG. 8.—Vaginal epithelium of old brown rabbit, iodined October 31st, 1892, to February 16th, 1893. ($\times 600$.)
- FIGS. 9-13.—Conjunctival epithelium from same guinea-pig as Figs. 1 and 2. ($\times 600$.)
- FIG. 14.—Vaginal epithelium of same rabbit as Figs. 3-7. ($\times 600$.)
- FIG. 15.—Vaginal epithelium of same rabbit as Fig. 8. ($\times 600$.)
- FIGS. 16, 17.—Vaginal epithelium of brown rabbit iodined for six months, and from June 19th to 23rd, 1893; scirrhous introduced June 23rd; animal killed June 26th, 1893. Foa. Ranvier's hæmatoxylin. ($\times 600$.)
- FIG. 18.—Vaginal epithelium of rabbit, iodined February 13th to 16th, 1893. Secondary epitheliomatous nodule introduced February 16th; animal killed February 19th, 1893. Foa. Ehrlich's hæmatoxylin. Enlarged from photograph magnified 400 times.

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FIG. 1.

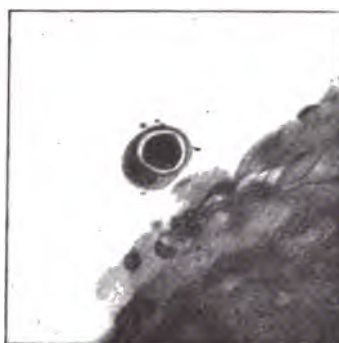


FIG. 2.



FIG. 3.

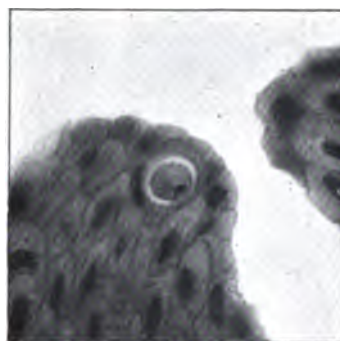


FIG. 4.

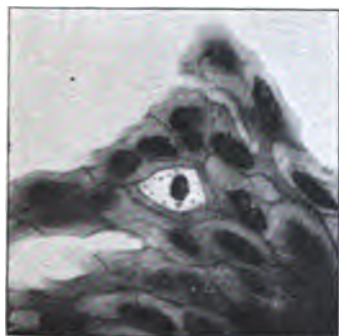


FIG. 5.

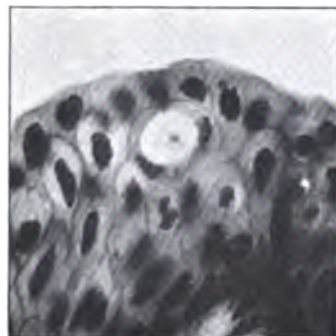


FIG. 6.

to you
ANGELIA



FIG. 7.

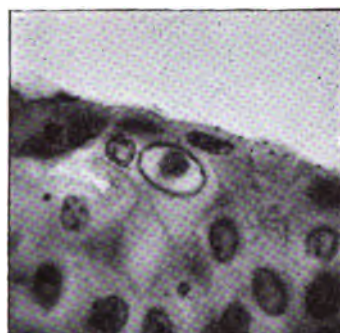


FIG. 8.



FIG. 9.

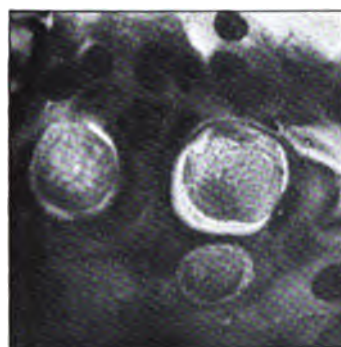


FIG. 10.

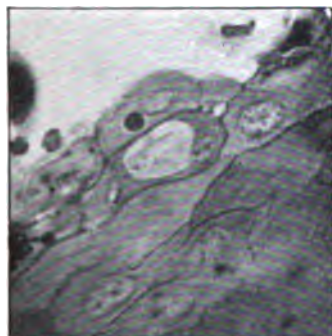


FIG. 11.

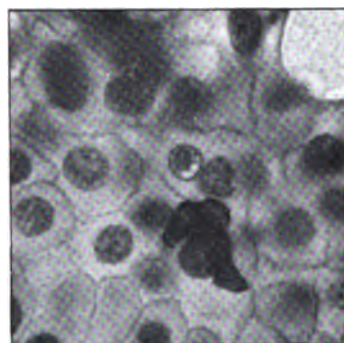


FIG. 12.



FIG. 13.

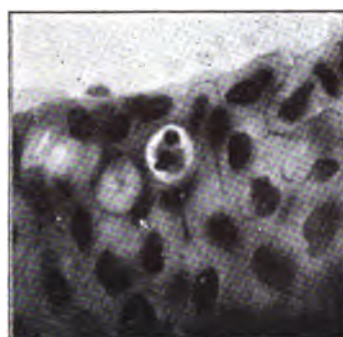


FIG. 14.



FIG. 15.

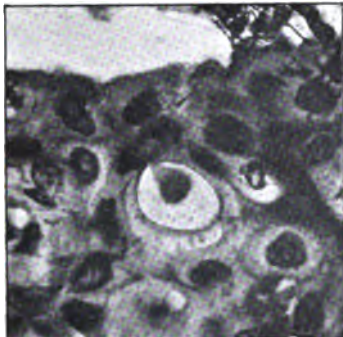


FIG. 16.

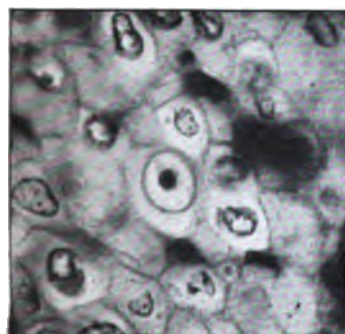


FIG. 17.

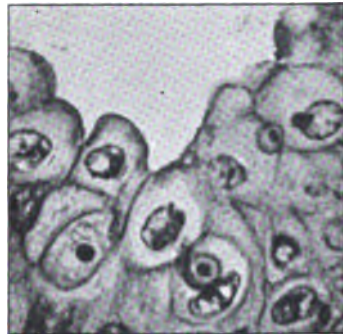


FIG. 18.

OBSERVATIONS ON THE ABSORPTION OF THE TADPOLE'S TAIL.

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(PLATE XI.)

It is well known that in the life-history of many animals, invertebrates as well as vertebrates, parts develop in order to perform a temporary function, and that such parts last only until others of a more permanent nature are developed. These permanent parts take on work of a similar kind, which, however, may be more specialised. In the tadpole, for example, the gills persist until the lungs develop and are capable of acting efficiently as respiratory organs; and the tail is maintained and remains active until the limbs grow sufficiently large to be capable of effectively propelling the small frog-like animal through the water.

The phenomenon of the disappearance of once functional parts, seen during the development of so many animals, is of interest not only to the biologist, but also to the pathologist; for the tissues composing the part that is disappearing undergo what may be called the normal, or physiological mode of degeneration and absorption. This process of degeneration and absorption of tissues in parts becoming functionless during development must indeed be the prototype of all the absorptive processes seen under so many different diseased conditions, and in all the various tissues of the body during childhood and in adult life.

The tadpole's tail is particularly suited to illustrate these processes, for it contains a variety of tissues, and is in great part composed of striped muscle in which the changes can be pretty easily traced.

The tail, for a time in the life-history of the tadpole, is the only motile organ, and by means of it the small creature moves briskly and merrily about in quest of food. As the limbs attain a proportionately large size, and commence to exercise their contractile powers, the tail begins to lose its motility, and becomes, at first slowly and afterwards rapidly, absorbed, until, after a comparatively short time, an interval of some days, nothing more than a mere stump, which ultimately dis-

appears, remains; and diminution in the motile power of the tail goes on *pari passu* with decrease in size, the former being due entirely, and the latter in great part (for muscle forms the main bulk of the tail), to changes in and subsequent absorption of the muscle fibres. By means of these changes, which affect the other tissues as well as the muscular fibres, the substance of the tail becomes transformed into portable material, which is conveyed either by the lymph and blood streams, or by some carrying agent or agents into the body, where it is disposed of in building up new tissues or eliminated by the excretory organs as waste product.

Metchnikoff¹ ascribes the absorption of the tissues of the tadpole's tail to the action of "phagocytes," which, he says, "are capable of incepting and digesting the individual tissues." This view is based upon the results obtained by observing the living tissues of the dwindling tail in a suitable medium and examining them under the microscope. He teased portions of the tail in aqueous humour and in serum, and found that in the teased tissue there were numerous large amœboid cells which were actively engaged in incepting and digesting fragments of both muscle and nerve fibres. The fragments so observed showed their natural structure before they had become altered by intracellular digestion. He does not explicitly state how the muscle and nerve fibres became reduced into fragments, but leaves us to infer that the process takes place through the medium of cells. Such phagocytic cells are numerous in the later stages of absorption of the tadpole's tail, and they are found chiefly among the fragments into which most of the muscle fibres break up. Before, however, the appearance of these *phagocytic* cells, other changes occur in the muscle fibres, which Metchnikoff, at any rate, does not mention.

Barfurth² has, since the publication of Metchnikoff's paper, given an account of the histological changes in the individual tissues of the tail prior to absorption. He points out that fatty degeneration of the muscle fibre, together with proliferation of nuclei within the sarcolemma, precedes the breaking-up of the muscle fibres into fragments which he calls *sarcolytes*, in contradistinction to *sarcoplasts*, a term which has been applied to similar bodies found in developing muscle. He also describes the phagocytes observed by Metchnikoff, and attributes to them the power of incepting and digesting the particles of altered tissue substance.

I propose to give here an account of some observations which I made in the early part of the past summer upon the tadpole's tail during its absorption, detailing the changes that occur in the individual tissues, and pointing out, as far as possible, the manner in which the altered tissues become absorbed and conveyed into the general system.

¹ "The Ancestral History of the Inflammatory Process," *Quart. Journ. Micr. Sc.*, London (New Series), vol. xxiv. p. 112.

² "Die Rückbildung des Froschlärvenschwanzes in die sog. Sarcoplasten," *Arch. f. mikr. Anat.*, Bonn, bd. xxix. s. 35.

The tail, which in the full-grown tadpole is about an inch in length, is composed of a central core, the notochord, surrounded by a urostyle, composed, near the body of cartilage, and away from it of a ring-like band of fibrous tissue enclosing the delicate tissue of the notochord, which is here relatively large. On the dorsal surface of this lies the spinal cord with its nerves on either side, upon each of which there are small ganglia, composed of several large typical ganglion cells. Enclosing the urostyle and the spinal cord is a thick layer of striped muscle which diminishes towards the extremity of the tail. This muscle is arranged like that in fishes; that is to say, it is divided by transverse or oblique partitions of delicate fibrous tissue into short lengths, the individual fibres being closely packed together, and ending abruptly, without any intervening substance in the fibrous partitions; investing the whole is a thin layer of areolar connective tissue, which is extended, both dorsally and ventrally, into a thin transparent fringe or fin, and the entire tail is, as well as the fins, covered, externally, with epidermis, consisting of three or four layers of cells resting upon a thin homogeneous fibrous membrane or cutis vera.

When the hind-limbs become capable of active movements, and when the fore-limbs are unfolding and project from under cover of the gill-plates, the tail begins to dwindle. The first change is a diminution in the ventral and dorsal transparent fringes or fins; then the central core with its muscle becomes less, especially near the extremity. In a day or two the whole of the fringes disappear, leaving a comparatively short tail tapering to a point. The tail now quickly becomes less, and its surface becomes somewhat ragged, as if pieces of the tail broke off into the water, until it is reduced to a small conical stump, which entirely disappears when the animal becomes a young frog, leaving, on the exterior, no trace behind. The stages in the changes in form of the tail and of the growing body are illustrated in Plate XI. Fig. 1.

THE MUSCLE OF THE TAIL.

The degenerative processes, which will be immediately described, begin simultaneously in the muscle fibres of different parts of the tail. For example, one muscle fibre here and another there may be found completely degenerated, while their neighbours are but little, if at all, altered. Thus the degenerating processes do not commence in the fibres at the very extremity of the tail and march upwards towards the root, attacking and destroying the muscle fibres in each successive compartment in which they lie; but they pick out, as it were, here and there, along the greater part of the length of the tail, those fibres possessed, perhaps, of the least resisting or vital power. Accordingly, the tail diminishes more or less uniformly in thickness throughout its entire length.

During this diminution in size, the following are the chief changes

observed in the muscle fibres. The fibre swells and becomes more or less homogeneous in its structure, the transverse striation being obscured. Subsequent to this, which may be called the first stage in the changes through which each fibre passes, one of two processes of degeneration may occur, according to the degree of rapidity with which the fibre disappears. If the process of degeneration is slow, the swollen fibre resolves itself into two constituents—the *one* a homogeneous material, which accumulates just within the sarcolemma and which is disposed in more or less regular rings joined in a variable manner; the *other*, a bundle of fibrils occupying the central part. These fibrils lie close together, but are apparently distinct and separable from one another; each fibril is transversely striated, just as in a normal fibre. The homogeneous substance would thus appear to be the protoplasmic substance intervening between, enveloping and binding the fibrils together. Thus the fibre may be said to be separated (1) into its primitive fibrils, which gather themselves together in the interior; and (2) into a homogeneous material, arranged in more or less annular bands lying just within the sarcolemma, and, as it were, binding the fibrils together as the binder does a sheaf of straws (see Plate XI. Fig. 6). Later, the homogeneous material disappears, and at the same time the fibrils break up into fine granules, which are probably, though I cannot definitely say so, composed of fat. While the above changes are proceeding, the nuclei of the muscle fibres, which are situated under the sarcolemma, proliferate, and form new cells that are set free when the fibre disappears. There are, however, no changes worthy of note in the surrounding connective tissue.

If, however, degeneration is rapid, the swollen muscle fibre, before it undergoes any further change, breaks up into minute oblong fragments of more or less uniform size. In some fibres, when this process of fragmentation is going on, small fat granules may be seen in the lines where the different pieces break off from one another, but in the majority of instances no such preliminary fatty change can be detected. These fat granules are not, I think, sufficiently numerous nor general enough in their distribution to warrant the view put forward by Barfurth, that prior to fragmentation the substance of the muscle fibre undergoes what he calls physiological fatty degeneration.

The fragments into which the muscle fibre breaks up are very minute, and, as a rule, of oblong shape, straight or curved, with rounded ends, resembling, as Barfurth says, small sausages; in many of them the natural transverse striation is seen, but in the greater number their substance is almost, if not quite, homogeneous. These fragments of the muscle substance are called by Barfurth *sarcolytes*, but a more appropriate name, if they deserve one at all, is *sarcomites*, seeing that the muscle fibre simply breaks up into minute fragments; and, to go a step further, the process of fragmentation would be called *sarcomitosis*. While *sarcomitosis* is in progress in the substance of the muscle fibre

the nuclei situated under the sarcolemma, as well as the nuclei between the fibrils proliferate and form new centres, around which new finely granular protoplasm is formed. These cells quickly increase both in size and numbers, and are to be found free between the sarcomites.

These are the cells described by Metchnikoff as *phagocytes*. They are best seen by teasing a piece of the interior of the living tadpole's tail, when it is one-half or even two-thirds reduced from its full size. This may be done either in serum, aqueous humour, or common salt solution, '6 per cent. These cells vary much in their appearances, and in their characters. Some are small, more or less globular, with large round nuclei and a small amount of very finely granular protoplasm (see Plate XI. Fig. 3, c). Others, which are most numerous in the later stages of absorption, are of large size and of irregular globular shape, the nuclei being obscured, and the protoplasm, which is abundant, loaded, we might well say "over-laden," with fat granules and fat globules (see Plate XI. Fig. 3, e). These large cells throw out small single or branched protoplasmic processes, the activity of which seems much increased a short time after the preparation of the specimen. The fat globules and granules are especially well seen in specimens treated with osmic acid. A third cell presents features intermediate between the first two kinds (see Plate XI. Fig. 3, d). These intermediate cells, which, so far as I have observed, are the most active amœboid cells, may be seen under the microscope ($\frac{1}{2}$ oil immersion) to throw out slender protoplasmic processes, so that at first the cell assumes a coarse prickly appearance, which suggested to Metchnikoff a resemblance to *actinophrys*. Some of these protoplasmic processes elongate and fork, as seen in Plate XI. Fig. 3, e. As I have said above, these are the only cells that show active amœboid movement, and some of these may be seen partially embracing the sarcomites, among which they lie, and into which the muscle fibre breaks up; but in the interior of none of them could I discern a sarcomite, although some fat globules were present. Moreover, in none of them did I find any recognisable piece or pieces of nerve fibre, as described by Metchnikoff.

In the protoplasm of these intermediate and active amœboid cells there are numerous fat granules, but in only a few are there fat globules.

Thus the cells found among the sarcomites of a rapidly degenerating muscle fibre may be classed under *three* forms:—(a) Small cells with large round nuclei and finely granular protoplasm which is small in amount—these cells resemble the leucocyte; (b) large globular cells of irregular shape, the nuclei obscured and the protoplasm over-laden with fat granules and fat globules; (c) cells of intermediate size which are capable of active amœboid movements, and, may be, of phagocytic properties. It seems, therefore, to be more than probable that the intermediate (c) cells, arise from the small cells (a), and that the large cells (b) arise from the intermediate (c); and I would make this suggestion, that these three forms of cells are merely different stages in the history of the one cell, which arises, in the first place, from the prolifera-

tion of the nuclei of the muscle cells lying within the sarcolemma, and of those lying between the fibrils. How far these cells are supplemented in their numbers by migratory white blood and lymph corpuscles, it would be difficult to determine, but it may, I think, be said that there is no evidence to be found in the tissues around of any migration of cells, or any indication to lead one to suppose that such does take place.

These cells, as I have said, lie within the sarcolemma and among the sarcomites; and as they increase in size and number the sarcomites diminish; so that, after a time, the latter disappear, leaving the cells, which are most of them large and laden with fat granules and fat globules, in their place. Ultimately the cells disappear, leaving no trace behind. Thus the cells, which are at first small, probably feed upon the nutritive material of the breaking up muscle fibres, and in consequence grow large, assimilating the food into fat granules—which later fuse to form globules—the fat becoming abundant, so as to completely obscure the nuclei of the cells. Then, it may be, that the cells thus laden with fat do themselves undergo disintegration, and so break up, the fat granules and globules being set free to be carried away by the lymph stream directly or indirectly into the vascular system, where they are made use of in building up new tissues, or oxidised and eliminated as waste product.

Do these cells feed after the manner of phagocytes, or do they, by secreting some digestive material, first cause liquefaction of the sarcomites, and then take up the products which they assimilate into their own substance? I have been unable to confirm Metchnikoff's observation that the cells enclose portions of the tissue—muscular, nervous, or other, which they subsequently digest, either in a teased portion of the tail in a .6 per cent. solution of common salt in water, or in thin serial sections, under the microscope. I found that the majority of the cells neither applied themselves to the substance of the muscle fragments, nor incepted any. Therefore, I am inclined to regard these cells as having the power of first secreting a digestive fluid capable of liquefying the sarcomites, and then of feeding and it may be of over-feeding themselves upon the products thus formed—the products being within their substance transformed into fat in the form of granules and globules.

With regard to the mode of origin of these cells, which are formed within the sarcolemma of the degenerating fibre, it may be noted that (1) all the above changes occur without any obvious vascular disturbances of the part, such as hyperæmia, which usually accompanies cellular activity, and (2) no accumulation of connective tissue cells, leucocytes, or wandering cells, takes place in the surrounding tissue. But the nuclei of the muscle fibres proliferate and form new cells, inasmuch as the nuclei themselves can be seen in various stages of division, and new cells resulting from this division may be seen clustered together. Besides, all the cells are confined within the sarcolemma and are nowhere else to be found.

It would appear, therefore, that these cells are most of them, if indeed not all, derived from the cells of the muscle fibres, those lying within the sarcolemma and those between the fibrils, and not from wandering cells, leucocytes, or fixed connective tissue cells.

To resume. As a rule, the degenerative process attacks the fibre, equally in its whole length, but occasionally the distal, and at other times the proximal parts, are first affected, and subsequently the process advances to the remainder. Of the first attacked muscle fibre nothing soon remains, and the neighbouring fibres, in which the process has not begun, come together; and in some, in which the process is incomplete, remnants of the muscle fibres may be seen, composed of longitudinal fibrils with irregular ends which in most instances have lost all traces of transverse striation, in which the proliferating cells above described are seen.

THE EPIDERMIS.

The epidermis, covering the full grown and as yet undiminished tadpole's tail, consists of 3 to 4 layers of cells. The deeper cells are subcolumnar, or cubical in shape, and rest upon a thin homogeneous fibrous cutis vera; the superficial cells are flat and scale-like and the intermediate polygonal. In many of the deeper and intermediate cells there are numerous black pigment granules, probably of the nature of melanin. Here and there the simple saccular mucous secreting glands of the epidermis are seen.

In the dwindling tail the epidermis is seen to undergo the following changes, which are especially well seen in tails reduced at least one-half in their size. At a little distance from the tip of the tail the epidermal cells are found increased to several layers, seven or eight it may be, the individual cells being small, polygonal, of nearly the same size, and in a state of active proliferation; the superficial cells are small and polygonal instead of flat scales, and the deeper cells are no longer of a subcolumnar shape. The formation of pigment in the deeper cells is much diminished, and the simple mucous glands are absent. Nearer the root of the tail the epidermis is seen to become gradually more and more normal, until the ordinary natural epidermis is reached; but on passing towards the tip the superficial layers diminish until near, and at the tip the epidermis is represented only, it may be, by a single layer of small polygonal cells in which no pigment is found. At the extremity of the tail the cutis vera, which is a nearly structureless layer of tissue, is almost absent, so that the epidermal cells on the exterior, and the connective tissue cells in the interior, border upon one another, there being often no distinct line of separation between them.

The epidermal cells, either when they proliferate, or when the deeper cells only are left after the shedding of the more superficial layers, show but little evidence of fatty degeneration in their protoplasm.

Therefore, during the disappearance of the tail, the epidermal cells, where active absorption is going on, proliferate and form new cells, which are small and of uniform size and shape. Such cells accumulate in several layers at first, then the more superficial cells become free and escape into the water, leaving, it may be, only one or two layers of small polygonal cells, which, owing to the disappearance of the cutis vera, lie next to the connective tissue cells of the interior of the tail. Ultimately, I presume, the remaining epidermal cells, together with contiguous portions of the tail, break off into the water and thus disappear.

Barfurth¹ says that in the dwindling tail the epidermis simply atrophies as it does in old age. My observations do not, however, confirm this view; in no stage of the absorption of the tail could this simple atrophy of the epidermal cells be seen. Rather, there is at first active proliferation of the cells, followed by a process of shedding, by means of which the cells escape into the surrounding water, with or without portions of the connective tissue forming the internal structure of the tail.

It may here fitly be asked whether any of the epidermal cells are absorbed through the interior of the tail into the body like the other tissues. All I can say is, that there is no evidence whatever to support such a view; but, on the contrary, all my observations point in the direction that the epidermal cells first proliferate and are then shed into the surrounding water, and so disappear.

THE SPINAL CORD, NERVES, AND GANGLIA.

In the tail portion of the spinal cord, as well as in the nerves proceeding from it and their ganglia, the processes of degeneration and absorption are not easily traced. Indeed, it would seem that these are the last structures to be affected, the cord appearing natural at a point where the greater number of the muscle fibres have already disappeared; and the nerves, even further back, can still be recognised in a state but little, if at all, changed. The changes in the cord that I have been able to discern are proliferation and increase of the cells lining the central canal, which is well developed, together with proliferation and increase in the number of neuroglia cells at the expense of the nerve substance of the cord; but in what manner the nerve substance disappears I have been unable to determine.

The proliferating cells of the central canal remain as last traces of the cord structure. The cells which are described above, as arising by proliferation of the cells of the neuroglia, have been regarded by Barfurth as leucocytes, but they differ from leucocytes in having smaller, perfectly round, and structureless nuclei, and an ill-defined outline.

¹ *Op. cit.*

In the nerves which run alongside the spinal cord, and which are medullated, it is difficult to ascertain more than that the nuclei of the cells are in different stages of division, the protoplasm remaining long unaltered. In the ganglia, however, the cells first become more granular and the pigment granules increase; while the cells themselves gradually diminish and ultimately disappear. The nuclei, however, which can long be readily recognised by their oval or ring-like outline with one nucleolus within, hold their ground to the last.

THE SPINAL COLUMN.

The prolongation of the spinal column into the tail is composed of a tube enclosing the notochord. This tube, seen in transverse section as a ring, if traced forward towards the body of the animal is found to be supported ventrally by a rod of hyaline cartilage. The walls of the tube are composed of almost transparent tissue (fibrous), having flattened connective tissue cells on the exterior.

During the removal of the tail, these cells proliferate and new cells are formed, which absorb and replace the fibrous matrix, and after a time become large and fatty. At the same time the notochord, which is originally composed of delicate branching connective-tissue cells, enclosing between their branches a mucoid, homogeneous matrix, is replaced by cells which assume a large size, and which are derived from the proliferation of the pre-existing cells. These cells also become very granular and fatty, the fat appearing in the form of granules.

Thus in both of these structures, spinal column and notochord, the cells of the respective tissues proliferate and form new cells, which cause absorption of the matrix and in time replace the tissue. When this is accomplished the cells grow large, granular, and fatty, after which they in some manner disappear.

Between the extremity of the spinal column and the tip of the dwindling tail there is a layer of tissue, composed in the main of fusiform and irregularly shaped connective tissue cells, amongst which remnants of muscle fibre and of other structures of the tail may be seen (see Plate XI. Fig. 2). The protoplasm of these connective tissue cells is abundant, highly granular, and in many instances laden with fat granules, the intervening matrix having disappeared. There is no line of distinction between the epidermic cells covering the tip and the connective tissue cells above mentioned, forming the subjacent part of the tail.

The fringes or fin parts of the tail, which are naturally composed of fibrous connective tissue, with but few connective tissue cells scattered in it, also undergo the same cellular transformation. Amongst these cells many small leucocyte-like cells may be seen, but no large giant or multinucleated cells of any kind.

THE CIRCULATION AND THE BLOOD VESSELS.

With regard to the circulation of the tail during its absorption, it may be said with certainty that there is no hyperæmia of any part during that process. Rather is the circulation diminished before any changes take place, the diminution in the circulation keeping well in advance of absorption. This may be well seen in the fins under a low power of the microscope. In the normal tail the capillaries run right up to the free margin of the transparent fin or fringe, where the circulation is as active as it is nearer the centre of the organ. In the early stages of the dwindling tail, however, the circulation does not extend up to the free margin, but fails at some distance from it; and at the line of failure outlying capillaries filled with stagnant blood may be seen. Thus the area of the circulation in the fins or fringes gradually shrinks, without any apparent cause, before the dwindling process sets in.

In the marginal part of the circulatory area some dilated capillaries may be seen containing stagnant red blood corpuscles, which gradually fuse, the vessels disappearing. Whether the change in the circulation depends upon contraction or any other alteration in the aorta, the leading vessel, I have been unable to ascertain.

RÉSUMÉ.

Before concluding this paper it may be well to draw up a short résumé of the changes observed by me in the different tissues of the tail during its absorption. In doing so I shall follow the same order as that in which they are described in the preceding pages.

Muscle.—The muscle fibres undergo two forms of degeneration. (1) The fibre swells and then resolves itself into a homogeneous substance, found immediately within the sarcolemma and into a central bundle of fibrils. This is the common form of degeneration in man and in the higher animals, after severance of motor nerves or destruction of the portion of the spinal cord. The homogeneous substance gradually disappears, leaving the fibrils which after a time break up into fine granules and which are thus lost. (2) The swollen fibre breaks up into minute sausage-like fragments, sarcomites, some of which show the natural transverse striation of the normal fibre, but most of which are, more or less, structureless. Among the sarcomites, cells which are derived from proliferation of the nuclei of the muscle fibre are soon found. As these cells increase in size and number the sarcomites diminish and disappear until ultimately only cells, often large and laden with fat in the shape of granules and minute globules, are left. After a time these disappear, leaving no trace of the fibre behind. All the muscle fibres disappear in one of these two ways, and when the muscle is gone its place is taken by a comparatively small amount of cellular connective tissue, in which the cells are prone to undergo fatty degeneration.

The epidermis.—The cells of the epidermis, like those in the interior, proliferate and form new cells, which are all of polygonal shape, small and of uniform size, the distinction between superficial and deep being lost. At first these cells accumulate and adhere to one another, so that the number of layers of cells in the epidermis becomes increased to 7–8 deep, at least twice as many as are found under normal conditions. Later, the more superficial cells fall off, leaving an uneven surface. This shedding, so to speak, continues, and after a time only a single layer of cells is left, which, it may be with contiguous portions of the tissue of the tail, drop off into the water. While this proliferation of the cells is going on they lose the power of forming pigment; thus the epidermis gradually loses its dark brown or almost black colour. Besides, in some of the cells, fatty degeneration of the protoplasm takes place. None of these cells are, so far as I have observed, absorbed into the tail and thence into the general system.

Spinal cord and nerves.—The changes that occur, both in the spinal cord and in the spinal nerves with their ganglia, prior to absorption, are not easily traced. Perhaps the most noteworthy observation is that these delicate structures persist unchanged longer than any other tissue. Indeed, it may be said that they are the last to become changed during this process of absorption. Further, the cells of the central canal, together with the ganglion cells, remain almost to the last.

The notochord and connective tissue.—The notochord, the prolongation of the vertebral column into the tail and the connective tissue, all undergo the same changes. These are (1) proliferation of the cells in each tissue, and (2) disappearance of the matrix, its place being taken by the newly formed cells, which after a time enlarge and become fatty. These cells are the last representatives of the structures of the tail, and they, in all probability, pass one by one into the lymph stream, and so into the general system.

Blood vessels.—While the above changes are taking place in the several tissues of the tail, the circulation becomes gradually and progressively diminished in its area, and maybe in the number of capillaries. This diminution continues until the absorption of the tail is completed. It is of interest to note that this diminution in the circulation is not due to a slow process of obliteration, say from proliferation of the lining endothelial cells, in the main artery of the tail; for nothing abnormal can be detected by the microscope in the wall of that vessel. At no time during the progress of absorption is there any undue vascularity (hyperæmia) of any part or structure of the tail.

REMARKS.

The results of the foregoing observations upon the absorption of the tadpole's tail show that, during the process, the following events succeed one another. *First*, there is (in the case of muscle) an alteration in the

matrix, whereby it becomes more amenable to the influence of its own and of surrounding cells; *secondly*, the cells proper to the tissue proliferate and increase in numbers, become active and acquire the power of throwing out amoeboid processes,—hence, they become capable of moving about,—and also the power of absorbing the altered matrix; and *lastly*, the cells, thus set free, grow large, become over-laden with fat, and presumably, after a time, disintegrate, set the fat free, and ultimately disappear in the lymph and blood streams. In this manner the tissues become ultimately transformed into cells over-laden with fat, which cells in due time disintegrate, and set the fat free to escape in the lymph and blood streams.

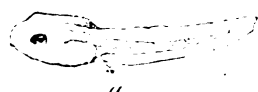
As I remarked in the introduction, the changes observed in the process of once functional parts are of interest, not only to the biologist but also to the pathologist; for these changes, seen in a pronounced degree in the tadpole's tail, are in all probability similar in kind, though greater in degree, to the changes that are constantly going on during the growth of the body from the early ovum, during the maintenance of the various tissues at their proper standard, and during the decay of the tissues in old age as well as in the different forms of atrophy observed under diseased conditions, and in the absorption of inflammatory and other products.

In the growth of the skeleton, parts are ever being formed, removed, and replaced, the formation of new parts exceeding the removal of the old. During adult life an almost even balance is maintained between the formation of new parts and the removal of the old, and thus but little, if any, change occurs in the form and structure of the bones during the period of adult life; in the aged, the removal of bone structure exceeds the formation, and the cells which determine this removal of bone matrix themselves become transformed into fat cells. A similar conversion of the protoplasm of cells into fat takes place in premature decay, for example, in the muscles of the heart, in the cells of the liver, etc.

In the removal of the excess of tissue after inflammation, after the healing of a fracture (temporary callus) and in the excessive scar-formation, similar processes, in all probability, occur, though they have not as yet been traced.

In cases of atrophy of bone from disease, for example, the absorption of the matrix and the conversion of the cells of the tissue into fat-containing cells take place as they do in the process of natural decay in the aged.

In degenerating new growths no change is so common as the transformation of the protoplasm of the cells into fat, in the form of granules and globules, together with the absorption of the intervening matrix. No better example of this can be found than in the case of slow growing scirrhus cancer of the breast; at the periphery of which the cells are numerous, healthy, and without any signs of fatty change



of their protoplasm; whereas, in the centre of which, the cells are few and their protoplasm loaded with fat granules and globules; in short, they are converted, before ultimately disappearing into fat-containing cells.

We see, therefore, that in the natural process of decay, as seen in the aged, in the premature atrophy from disuse or disease, in the removal of excess of tissue and in the degeneration of new growths as well as in the physiological and embryological processes, the tendency on the part of the cells of the tissue is to become transformed into fat before they disintegrate and ultimately disappear, their contents thus escaping into the lymph and blood streams, where the fat is either used as food, just as is ordinary fat absorbed from the alimentary canal, or eliminated as waste product. I hope, in a subsequent paper, to enter more fully into this very interesting subject.

DESCRIPTION OF PLATE XI.

FIG. 1.—*a*, *b*, *c*, and *d* show the stages in the disappearance of the tadpole's tail, and the transformation of the tadpole into the frog.

FIG. 2.—Transverse section of the tadpole's tail, when dwindled to one-half its natural size. ($\times 40$.)

- (*a*) Vertebral column.
- (*b*) Spinal cord.
- (*c*) Natural muscle fibres.
- (*d*) Degenerating muscle fibres.
- (*e*) Thickened epidermis.

FIG. 3.—Cells obtained by teasing a portion of the muscular substance of the half-dwindled tail in '6 per cent. solution of common salt. $\frac{1}{4}$ Oil-immersion. ($\times 700$.)

- (*a*) Red blood corpuscle.
- (*b*) White blood corpuscle.
- (*c*) Small cells.
- (*d*) Intermediate-sized cells.
- (*e*) Large cells.
- (*f*) Sarcomites.

FIG. 4.—Muscle fibre from the half-dwindled tadpole's tail, showing the splitting up of its substance longitudinally. ($\times 400$.)

FIG. 5.—Muscle fibre broken up into small blocks (sarcomites), some of which (*a*) show the natural transverse striation, but the majority (*b*) are more or less homogeneous in their structure. ($\times 450$.)

FIG. 6.—Muscle fibre, the substance of which is separated into (*a*) a central bundle of fibrils, and into (*b*) a homogeneous substance in the form of rings lying around the bundle of fibrils and within the sarcolemma. ($\times 450$.)

A RARE MORBID CONDITION OF THE URINARY BLADDER (FIBROMYOMATOUS CHANGE).

By T. K. MONRO, M.A., M.B., *Assistant Physician to the Royal Infirmary
and Pathologist to the Victoria Infirmary, Glasgow.*

(PLATE XII.)

THE following case is of interest, chiefly on account of the remarkable appearance presented by the wall of the urinary bladder, the condition being, probably, of congenital origin.

The patient, a commercial traveller, æt. 29, was admitted to the Victoria Infirmary, under Mr. Maylard, on the 21st February, 1893. He complained of pain and difficulty in micturition. From early life, he had to strain while emptying his bladder. Ten years before admission, he had a slight illness (supposed to be due to hepatic disturbance) associated with a high colour of the urine, which threw down a sediment on standing. He afterwards went to Wales for a holiday, and while there, injured his left buttock.

The illness for which he now sought advice began 2 years before, the complaint being of severe pain in the left lumbar region, passing downwards to the left groin and testicle, and accompanied by sickness and vomiting. These symptoms would pass off after 2 or 3 days, to recur after an interval varying from 10 days to a month. In the early part of the illness, clotted blood was observed to be present in the urine for 2 or 3 days. In spite of his ill-health, the patient continued to attend to his business. There was nothing worthy of note in the family history.

On admission, a hard, tender swelling, dull to percussion, was found in the lower part of the abdomen. There was also a sense of tumour in the left lumbar and iliac regions; and at the upper part of this tumour there was felt a hard, egg-shaped body, which could be rolled under the fingers. Another and more superficial body was detected in the same neighbourhood.

The urine was decomposing and contained abundant albumen; but no tube casts or blood corpuscles were observed. There seems to have been no difficulty whatever in passing an instrument into the bladder.

As this case is recorded on the ground of its morbid anatomy, and not on account of its clinical features, it may be briefly stated that, in the course of an operation undertaken to relieve the symptoms, it was

deemed advisable to excise the bladder altogether. The operation was a tedious one, as the ureters—to mention only one disturbing element—were so much dilated as to resemble small intestines; and when these were cut across they poured out a huge volume of urine into the wound. The patient died 5 hours afterwards.

A complete post-mortem examination was not made, but the following structures were removed and given to me for investigation :—

1. The bladder, with
2. Certain bodies seated on its upper end ;
3. The kidneys and ureters ; and
4. Two isolated tumours said to have been connected with the pelvis.

(1) The bladder was greatly enlarged, and its wall was much thickened. The inner surface of the organ suggested the appearance of a lobulated kidney, but the lobules were irregular in shape and size, some being very large. The depressions between the lobules were almost $\frac{1}{2}$ in. deep ; whilst one such—situated to the left of the trigone—reached actually to a depth of more than $1\frac{1}{2}$ in. below the general surface. The lobules—as regards their surface—were of a reddish-brown colour, with a tint of blue, but numerous white patches were seen upon them ; these consisted of flakes of epithelium, and were easily detachable.

The lobules, or elevations of the mucous surface, were found on microscopic examination to consist of unstriped muscle and of connective tissue elements. There was, moreover, an abundant infiltration of round cells, which, at some parts, were collected in such numbers as to suggest the commencement of small abscesses. This last-mentioned appearance may perhaps be connected with the desquamation or loss of epithelium already alluded to.

(2) The bodies on the upper end of the bladder presented, to the naked eye, the appearance of convoluted rolls or worms embedded in fibrous tissue. Microscopically, they were found to consist largely of unstriped muscular fibres. Thus, one of the rolls cut across might show an investment of unstriped fibres coursing round it in a ring, the central portion or axis of the roll being constituted by similar fibres running in the direction of its length. These rolls also contained fibrous tissue and numerous capillaries. In some places, branched cells of irregular form could be seen ; also cells resembling epithelium, but devoid of any apparent nucleus.

(3) The kidneys and ureters were such as are seen in fully developed hydronephrosis. The left kidney was 8 in., and the right 6 in. in length. On the left side, two ureters passed down from the kidney to the bladder. These were at first quite separate, but they became united to one another half-way down, though the lumen of each remained distinct from that of the other throughout its whole length, and the two opened by separate orifices into the bladder. The right ureter was single. All three ureters were much dilated, and measured, at parts, when lying flat on the table, more than $1\frac{1}{2}$ in. across.

(4) The two tumours connected with the pelvis were typical hard fibromata. They measured about 2 and 3 in. in length respectively. Each was ovoid in form, with a distinct capsule, and varied in hardness in different parts. The cut surface was white and glistening, and the microscopic appearances were those of fibrous tissue with abundant spindle shaped nuclei and occasional blood vessels.

REMARKS.—There can be little doubt, I think, that this abnormality of the bladder wall was of congenital origin, though probably, like a *nævus*, it may have been a slight thing at the time of birth, and may have developed greatly as the patient grew up. The hydronephrosis

was no doubt due to the condition of the bladder. One of the large lobules on the inner surface of the organ corresponded closely in situation to the trigone. One ureter opened to the right of this; the two others to the left. The orifice of one of these latter was situated deeply on the side wall of the very deep interlobular furrow described above. Obviously, therefore, the ureters would be continually liable—probably continually subject—to very considerable pressure, as they passed through this great thickness of bladder wall. The pressure on the ureters, and the consequent obstruction to the flow of urine, would become all the greater, if, as is likely, the increase in thickness of the wall was progressive.

The condition of the bladder, the existence of two ureters on the left side, the convoluted bodies on the upper end of the bladder, and the two fibromata, are four facts which together point to a strong tendency to abnormality in the body of this individual; while the clinical history seems to show that the malformation of the bladder had already begun to produce its effects in early life. We must, therefore, suppose that the hypertrophy was of congenital origin, or due to a congenital tendency.

Owing to the incomplete nature of the autopsy, or more probably to a regrettable oversight, there is no record of the urethra having been specially examined after death, with a view to ascertain whether it presented any condition likely to cause obstruction to the outflow of urine. But, as has been already stated, there seems clearly to have been no obstruction to the passage of a catheter into the bladder during life. While, therefore, it is conceivable that there may have been some abnormal structure of a valvular character, interfering with the emission of urine, it appears to me that a theory of this kind is not required to explain the facts, which may quite well be accounted for by the state of the bladder itself.

Fibromata and myomata are very uncommon in connection with the bladder, and, when they do occur, take the form of more or less definite tumours. In this case, however, there was what might be termed a fibromyomatous change, involving the bladder wall generally, but showing chiefly on its mucous surface. I have consulted a large number of the most authoritative works on diseases of the bladder, but have been unable to find any record of anything similar to what has been here described.

My thanks are due to Mr. Maylard for his kind permission to publish this case, and for information with regard to its clinical aspects.

DESCRIPTION OF PLATE XII.

FIG. 1.—Kidneys and ureters. The right kidney is cut open; the left gives origin to two ureters.

FIG. 2.—Bladder cut open in front. (Unfortunately the original negative taken from the fresh specimen went amiss in the engraver's hands. The present illustration was obtained from a negative taken from a water-colour drawing.) The lobulated character of the inner surface of the organ is seen; also the greatly thickened wall, and the orifices of two of the ureters.

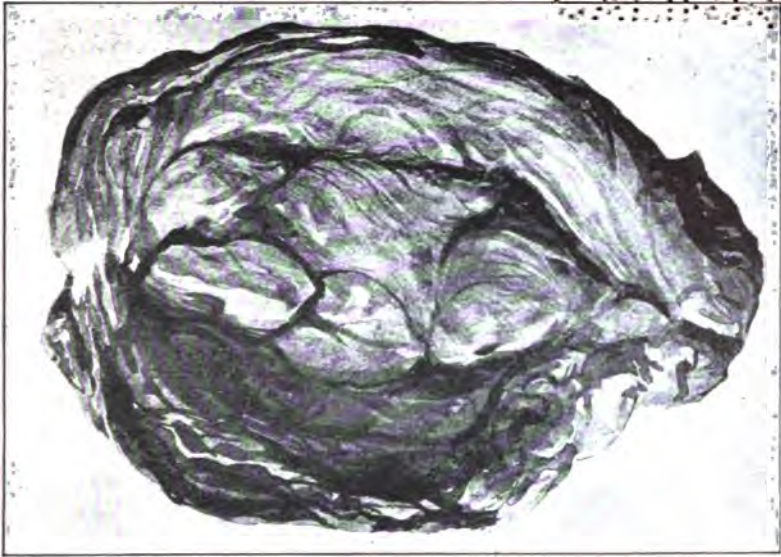


FIG. 2.



FIG. 1.

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AN APPARATUS FOR RAPIDLY INFILTRATING WELL DEHYDRATED TISSUES WITH PARAFFIN.

By G. L. CHEATLE.

VARIOUS methods of bringing about the infiltration with paraffin, of perfectly dehydrated tissues, have from time to time been suggested.

The one in use at the laboratories of the Royal College of Physicians (Lond.) and Surgeons (Eng.), where this method has been used for some time, consists of a series of tubes to which rubber corks, to take specimen or test tubes, can be fitted. These, placed in a hot-air chamber in a basin of oil so that the paraffin may be kept melted, are connected with an exhaust pump, by means of which the chloroform vapour may be readily drawn out.¹

The modification I now suggest pretends to nothing new in principle, but I think, after careful trial of its merits, that it will commend itself to those engaged in histological investigation.

The objects of this apparatus are:—

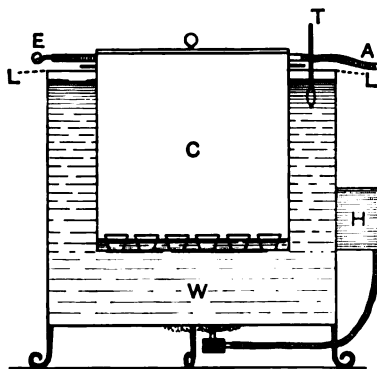
1. To ensure the embedding in, or infiltration with, paraffin of tissues *in vacuo*, or under diminished atmospheric pressure.

2. To effect this at a constant and invariable temperature, the temperature being 2 or 3 degrees only above that of the melting point of the paraffin used.

3. To keep the capsule or capsules (in which the tissues are soaking in paraffin) in contact with a fluid maintained at a constant temperature. This third point is laid great stress upon by Mr. Charles Hearson, and other experts.

The following brief description of the apparatus explains the methods by which these objects are attained.

A tank W is filled with water, which is maintained at a constant temperature by means of a "Hearson" regulator H. An inner tank C, surrounded by the water contained in W, has on its floor a layer of olive



¹ Pringle, *Journ. Path. and Bacteriol.*, Edin. and London, vol. i. p. 117.

oil, or glycerine, or even melted paraffin. In this are placed the capsules holding the tissues to be embedded. This tank is closed in at the top by a disc of plate glass. Communicating with the inner tank is a tube A, which is connected with an air pump. On the opposite side is a screw E, by means of which the entrance of air to the inner chamber may be regulated, or cut off. Between the outer and the inner tanks is a space, the top of which, covered in, forms a ledge L, on which sections may be flattened out in a satisfactory and reliable manner, by placing them on water on slides, on which they are exposed to the temperature maintained in the water of the outer chamber T, a thermometer indicating the temperature attained in the water.

For the method of using the apparatus, I may refer readers to Mr. Pringle's note on "Paraffin Infiltration by Exhaustion," which appeared in vol. i. p. 117 of this *Journal*.

The whole apparatus is constructed of copper. It is very compact, and gives most accurate results.

For this scientific accuracy I have to thank Mr. Charles Hearson, who has afforded me most valuable advice and assistance in the planning and construction of my apparatus.

A SPECIMEN OF THE SO-CALLED SIREN-MALFORMATION (SYMPUS, SYMELIA).

By JOHN H. TEACHER, M.B., C.M., and JOSEPH COATS, M.D.

From the Pathological Laboratory of the University of Glasgow.

THE malformation, of which the subject of the present paper is an example, receives its most usual name of *Siren-malformation*, or even *Sirenia*, from the resemblance in general form to the mythological inhabitants of the sea, whose fatal charms were supposed to be exercised on sailors. The specimen is a typical one, and occupies a middle position between the more extreme and less pronounced forms.

For the specimen we are indebted to the kindness of Dr. John Adams of Glasgow, who gives the following account of the circumstances connected with the birth of the child :—

“The mother is a healthy woman about 32 years of age, with a good family history and also a good record of previous health. She has borne three children at the full time, all well developed and healthy at the time of birth. She has had no miscarriages. In the course of the present pregnancy, no extraordinary feature of any kind occurred. Movement was felt when the pregnancy was advanced about $4\frac{1}{2}$ months. There is no story of any maternal impression, nor of any fright, nor of any misshapen individuals being seen or imagined during the whole period of gestation.

“Labour began on a Friday afternoon with slight pains at long intervals—an hour or so at first. These pains continued till the Monday morning, when I was called about three o'clock to find the woman running about the house and not seemingly in any great distress. She was getting anxious, as she was not aware of making any progress. The membranes were said to have ruptured during Sunday afternoon.

“She was put to bed and examined. The presenting part gave the idea at first of a breech, but further investigation did not confirm this, as no furrow between the buttocks or anal orifice could be discovered. The ease with which a foot could be reached led to the conclusion that the presentation was one of a knee. The pelvis being roomy and the patient's strength good, it was resolved to attempt delivery without turning. With the forefinger of the right hand in the flexure of the knee, and with the left hand exercising friction and pressure on the abdomen, strong pains were induced, and the product of conception was expelled in about fifteen minutes. The foetus had evidently been dead for three or four days.

“The woman made a good recovery.”

The foetus as received at the laboratory was described and figured

before any dissection was attempted. The viscera were then examined and removed, and afterwards part of the skeleton was dissected out. The external parts were now restored as nearly as possible, and the foetus as a whole and the parts of the skeleton are preserved in the museum of the Western Infirmary, Glasgow.

The general aspects of the foetus are shown in Fig. 1, which

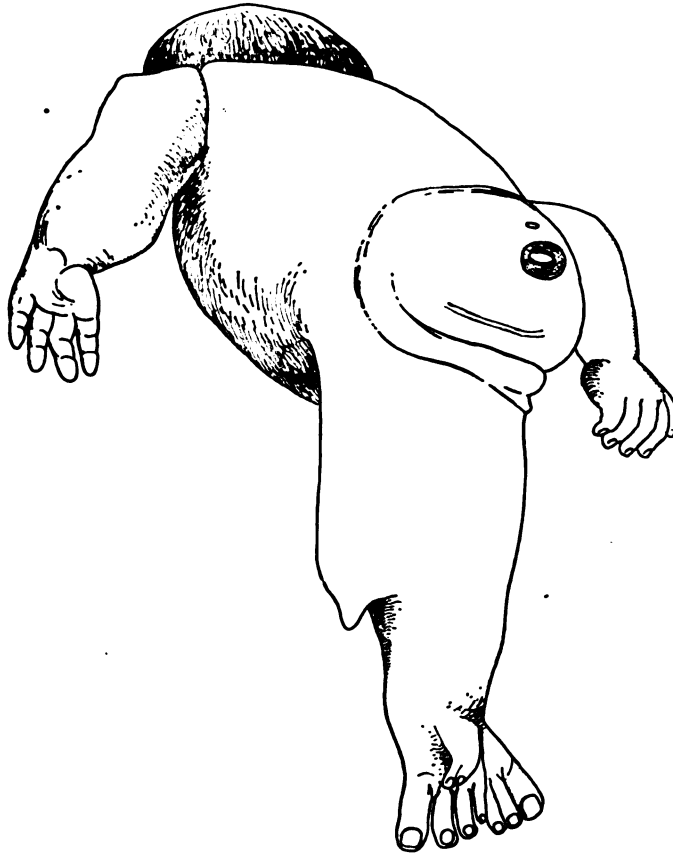


FIG. 1.—View of foetus from behind. The limbs coalesce and end in a single foot, having seven distinct toes and two coalesced ones higher up. The two great toes are at the outer aspects of the foot. The dorsal aspect of the foot is that shown, and it faces backwards. There is one rounded buttock with a large anal opening. The left hand has four fingers.

represents a posterior view. The trunk ends in a single rounded buttock, near the upper part of which is the anal aperture. The two lower limbs are coalesced into one broad member, which tapers downwards and ends in a broad foot, not unlike a fish's tail, furnished with nine toes. The left hand has only four fingers.

Making the examination more in detail, it was found on handling the foetus that it could be very readily doubled up anteriorly, there being

exceedingly free movement between the trunk and lower extremity. This movement was obviously not at the hip-joint, but higher up, as the pelvis moved with the lower limb. The seat of this loose connection was found on dissection to be the sacro-iliac articulation.

The aperture in the upper part of the buttock was obviously the anus pushed backwards and upwards. It admitted a good-sized quill, and a probe could be passed several inches into the rectum. Immediately above the anus there is a small dimple in the skin (see Fig. 1), and the tissue here was cicatricial in appearance. The dimple seemed to be in connection with the tip of the coccyx.

Viewed anteriorly no proper genital organs were visible. The only indication was a small projection about the size of a good-sized pin's head, situated in the middle line, 6.5 cm. ($2\frac{1}{2}$ inches) below the umbilicus.

The single lower limb has evidently arisen by a coalescence, in some places by no means complete, of the two ordinary limbs. But the coalescence has not occurred by the aspects which are nearest one another in the fully developed legs. It is as if the limbs before coalescence had been so placed that their outer aspects were in the middle line behind and had united and become fixed there. Thus the normal outer borders of the limbs are merged together in the middle line, and their structures partly suppressed, while the inner have become the outer borders. In this way the hip-joint is behind; the knee is reversed and flexes forwards instead of backwards. The plantar aspect of the foot presents forwards, and the great toes are at the outer borders of the foot.

Entering more into detail, it could be felt before dissection that the double femur was, especially at its lower part, unduly broad, and that it had a double lower extremity. The single foreleg was attached to the right half of the coalesced femur, and there was an abortive left foreleg, 2 cm. in length and tapering to a point, attached to the articular surface of what represented the left femur.

The foot was loosely articulated at the ankle, and its plantar aspect presented forwards. The foot was an almost direct prolongation downwards of the leg. It ended in a row of seven toes (see Fig. 1), of which the two outer are the great toes, and, of the remaining ones, three seemed to belong to the right foot and two to the left. In addition there were visible, on the dorsal aspect, two small coalesced toes, forming a broad but short double toe with two small nails. There are thus, in all, nine toes represented. The width at the ankle was 2 cm., whilst across the outspread toes the breadth was about 4 cm.

The upper limbs were well formed, with the exception that the thumb of the left hand was wanting, and the only indication of it was a small projection, oval in shape and the size of a pin's head.

On opening the body the following conditions of the intestine were found, there being three complete occlusions of the calibre of the gut, two of them with entire interruption of the continuity.

Taking the structures from below upwards, it was found that the anus communicated with a well-developed rectum, and that the descending colon was apparently normal. This part of the intestine terminated above in a rounded blind end, which was situated about 16 cm. above the anus. Examining further, the intestine was resumed on the right side by a bulky blind end, which represents the upper part of the ascending colon or transverse colon. To the apex of this a narrow tendinous band was attached, representing collapsed and occluded ascending colon. This being traced downwards there comes first a small finger-like diverticulum, 1.5 cm. in length, and then a narrow caput cæcum with vermiform appendage and ileo-cæcal valve. These are about 23 cm. from the upper blind end of this part. From the ileo-cæcal valve the small intestine was only traceable upwards for a distance of 11.5 cm., and here it suffered a complete interruption with a rounded blind end. There were still abundant coils of small intestine, but their relations could only be made out by tracing the parts downwards from the stomach. The stomach itself was found to be normal, and the small intestine was continuous through the various coils till it ended in a dilated and somewhat infiltrated piece of intestine.

It will thus be seen that the great intestine has been completely cut across, apparently in the situation of the transverse colon, possibly with removal of a portion of the colon. The two ends produced by the lesion have formed rounded bulbous extremities. Then, also, the small intestine a short distance above the valve has undergone a precisely similar interruption. In addition, the ascending colon has had its calibre abolished over a considerable extent, but without absolute severance of its continuity.

The two kidneys were found in their normal position. The testicles were also found lying in the abdomen and apparently normal. The liver and spleen were also apparently normal.

The *skeleton* was removed from the second lumbar vertebra down to the ankle-joint, and, without further dissection, the following points were noted (see Figs. 2, 3, and 4). The sacrum was very loosely articulated to the iliac bones, and it was due to this fact that the pelvis and lower extremity could be so freely doubled up on the abdomen. The most prominent peculiarity of the pelvis is the fact that the ischial bones have coalesced across its floor (see Figs. 2 and 3). Behind the coalesced ischial bones the rectum passed out, being thus pushed backwards and upwards. This accounts for the peculiar position of the anus. The coccyx and sacrum were tilted backwards (Fig. 2).

A more detailed examination of the portions of the skeleton removed gives the following results, which are illustrated in the sketches on the opposite page.

The parts removed are these: the last three lumbar vertebræ, the

pelvis, one large bone representing both femora, the tibia and fibula of the right leg and both patellæ.

The three vertebræ were found to be normal in front. They are closed behind, so that there is a complete spinal canal, but the laminae are cartilaginous, and there are no spinous processes. Fig. 3, which



FIG. 2.



FIG. 3.

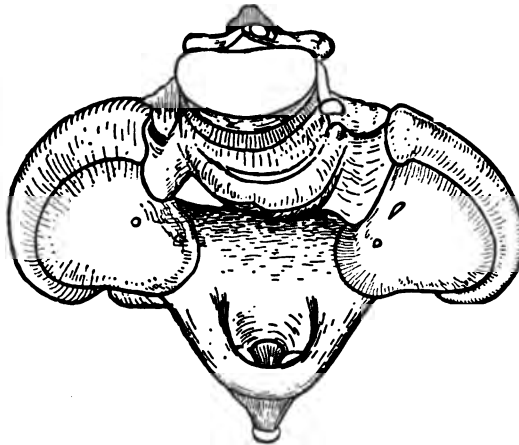


FIG. 4.

FIG. 2.—Part of the skeleton seen from behind. The broad femur has a double head, which articulates with the pelvis behind. The patellæ are shown, the left looking backwards, the right backwards and outwards.

FIG. 3.—Three lumbar vertebræ and sacrum seen from behind. The arches of the vertebræ are incomplete, and there are no spinous processes. In the sacrum the vertebral canal is completely open.

FIG. 4.—Pelvis seen from the front. The ischial bones are coalesced across the floor of the pelvis. The pubic bones are also coalesced and are projected forwards.

represents the posterior aspect of the vertebræ and sacrum shows this. The sacrum, as shown in Fig. 2, is bent backwards at right angles to the vertebral column, and is also slightly twisted towards the right side. In the sacrum (see Fig. 3), the vertebral canal is completely open, two or three tubercles at each side representing the laminae.

The iliac bones seem to be well developed. They diverge widely,

and they are somewhat rotated, so that their anterior borders look directly downwards. The lines of junction with the ischia are well marked in front.

The ischial bones are markedly altered, being transformed into a thick bar of bone which crosses the pelvis nearly parallel to the anterior surface of the sacrum (see Fig. 4). From this project forwards three processes, which meet in the middle line in front. The two lateral ones, which represent the horizontal rami of the pubic bones, lie higher than the third, and form a rounded projection anteriorly. The third, which seems to represent the two descending rami of the pubic bone united into one, is in the middle line, and passes from the middle of the coalesced ischia to the pubis in front. This median process was separated from the lateral ones on either side by a small aperture, and these apertures represent the obturator foramina. To the lower border of the median process was attached a rudimentary penis.

Between the bar of bone representing the ischia, and the sacrum, is the pelvic outlet, which is much narrowed from before backwards. It measured 2.25 cm. transversely, and from before backwards .5 cm. at the left, and .7 cm. at the right side (see Fig. 2).

On the posterior aspect of the pelvis, just at the junction of the ilia with the transverse bar, are two acetabula, looking backwards and slightly downwards. The synovial cavity of each is quite distinct.

The two pubic bones are coalesced in front and projected considerably forwards (see Fig. 4). The horizontal rami are separately distinguishable, but the descending have coalesced into the single process, which, as already mentioned, joins the bar formed by the coalesced ischia. The infrapubic arch is thus abolished.

The two femurs are represented by a single shaft, which is bifid above and below (see Fig. 2). Above, there are two distinct heads articulating separately, two necks, and two great trochanters. The shaft itself is markedly flattened and broad, measuring 1.2 cm. at its narrowest part.

The lower end bifurcates a fifth from its extremity, and there are two distinct and separate articular ends, each having the proper surfaces for the knee-joint and its own patella. The position of the articular surfaces in relation to the normal, is altered in accordance with the rotation of the limb already mentioned. This is indicated by the position of the patellæ (see Fig. 2), the left patellæ looking almost directly backwards, and the right backwards and outwards. The right knee-joint is completed by the tibia and fibula. The left was formed by the aid of a bone which was left in the body. There is a fibrous separation between the two ends of the femur.

The tibia and fibula are normal except at the lower extremities, which are very slightly altered so as to articulate with the deformed foot.

Regarding the bones as a whole, the extreme flexibility of the lower extremity forwards is explained by the looseness of the sacro-iliac

synchondroses, by the position of the hip-joints, which prohibits backward movement but allows of considerable flexion forward, and by the reversal of the knee so that it flexes forwards instead of backwards.

REMARKS.

The specimen described above conforms in most respects to the cases of a similar lesion described by various authors. There are differences in the degree of coalescence of parts in the recorded cases, but in their general features and in the kind of defect in the pelvis and leg there is a close similarity.

Taking the classification of such malformations adopted in the exhaustive work of August Förster,¹ which, with differences of nomenclature, is virtually that of Geoffroy Saint Hilaire,² we find that our specimen belongs to the general class of *Sympus* or *Sympodia*, signifying a coalescence of the lower extremities. Geoffroy Saint-Hilaire uses the more general term *Symèle* (μῖλος, a limb). The class is divisible into three groups which, to some extent, merge into each other but are sufficiently distinct for practical purposes. There are first the cases in which the defect is greatest; the foot is absent altogether, and the leg ends in a rounded or tapering extremity. This is the *Sympus apus*. Geoffroy Saint Hilaire limits the term *Siren* to this form, and designates it *Sirénomèle*. The second group is that to which our case belongs. There is such a coalescence that there is only one foot, although the toes may be more numerous than those of the normal foot. This is the *Sympus monopus*, or the *Uromèle* (οὐρά, a tail). In the last group there are two distinct feet, and this form is called *Sympus dipus*, or by the general term for the whole group, *Symèle*.

The peculiarities which are common to the whole group, and which give it its unity, may here be enumerated, as they are illustrated in our case. It seems to us that the central item in the defect is the condition of the pelvis and especially of the ischia. In Förster's very careful descriptions of the three forms, the coalescence of the ischial bones is the only absolutely constant lesion of the skeleton. In our case the two ischial bones form a transverse bar across the pelvis, and this seems to be the regular condition. Even in the least advanced cases this feature is present. There is a case of *sympus dipus*, for example, related and illustrated by Cruveilhier,³ and given also in the Atlas to Förster's work, in which all the bones of the leg and foot are doubled, and the only coalescence is between the two calcanei; but the pelvis, as appears from Cruveilhier's description, and his most careful illustration, is very much like that in our case. The two ischia are united, and the

¹ Förster, "Die Misbildungen des Menschen, nebst Atlas." Jena, 1865.

² Geoffroy Saint-Hilaire, "Histoire générale et particulière des Anomalies, avec Atlas," Paris, 1832.

³ Cruveilhier, "Anat. Pathol.," livr. 40, plate vi.

pubic bones are pushed forward prominently, in a similar position to that shown in Fig. 4.

Another constant feature is the altered rotation of the limbs, which is so fully illustrated in our case, and this connects itself directly with the central lesion just mentioned. It seems to be a common feature that, when the bones of the leg coalesce, they are united by their external borders, which have been rotated backwards and inwards. Even in cases of dipus, where the whole of the bones of the leg are doubled, the fibulae are to the inside and the tibiae external; and the great toes are at the outer margins of the double foot. This peculiarity is to be related to the coalescence of the ischia, which we have regarded as the central lesion. The coalescence of the ischia, in the middle line behind, displaces the two hip-joints backwards, so that they approximate to each other, or even coalesce posteriorly. In this approximation of the acetabula, there is necessarily a rotation of these articular surfaces. The acetabula, instead of looking outwards and forwards on each side of the pelvis in the normal fashion, will, in proportion to their approximation to the middle line behind, come to look more and more backwards. Following on this, the femora will be similarly rotated, so that their articular surfaces, instead of presenting, as in the normal hip-joint, inwards and backwards, will come to project according to the degree of the lesion, more and more directly forwards. This will bring into close proximity the posterior and outer aspects of the femurs, and cause them to be fixed in this rotated position.

The degree of the lesion will be determined by the extent of the coalescence of the ischia, and the consequent approximation of the acetabula behind. There may be such an approximation of the acetabula as to make only one joint cavity, and that a shallow one; and this coincides with the extreme forms of the defect in symplus apus. On the other hand, there are the various degrees of two acetabula with united femora (as in our case), and a single foot, on to those in which the acetabula, being considerably separated, the bones of the leg and foot are completely doubled. It will be obvious that it is this rotation of the femurs, and their approximation by their posterior and outer borders in the middle line behind, that brings about the rotation of the leg as a whole; the abnormal flexion of the knee-joint, and the position of the great toes externally, and the little toes internally.

Lesions of the terminal parts of the intestinal and urino-genital apparatus are apparently constant in all forms of symplus. It might be expected that the coalescence of the ischia, directly across the position of the anus, would be liable to abolish that orifice and the last part of the rectum. It has not done so in our case, but this seems to be an entirely exceptional circumstance. Imperforate anus is stated as a constant feature by Förster, who says, in his account of the whole group, "Der After fehlt stets." In our case the anus was pushed backwards, but the rectum and outlet were well developed. The three in-

terruptions of the calibre of the intestine, however, were equally effective in rendering the continuance of life impossible, even if the child had been born alive.

The urino-genital organs are also in all cases interfered with. There may be no appearance of external organs, or a mere trace as in our case, or an approximation to the normal genitalia, but it is stated that there is never any opening of the urino-genital passages externally. Whether this is liable to exception, as in the case of the anus, remains to be seen. It is stated by G. Saint-Hilaire that the kidneys, at least on one side of the body, are always involved. In this respect also our case is an exception, as both kidneys and both testicles were present and apparently unaffected.

We come now to the question of *the origin and causation* of this form of monstrosity. In view of what has been said as to the central point of the defect being in the floor of the pelvis, we have to consider what kind of lesion may have produced it, and at what period of development. The lesion is not a mere error in development. It is a lesion, having certain definite and constant characters, which are all traceable to a defect in a particular part of the fœtus.

The lesion must have originated at an early period of utero-gestation. The formation of the buds which go to form the lower limbs must have just begun. There was no differentiation as yet of the individual bones in any part of the limb, except, it may be, of the digits. The buds forming the lower limbs are placed at first as flaps against the lower part of the body of the embryo. These flaps, at first, represent little more than the foot, whose elements are the first to appear, and they are applied to the body, so that the great toe is towards the cephalic end of the fœtus, and the little toe towards the caudal end. In this early period, should some agent acting on the posterior parts of the embryo, where the primordial pelvis is situated, cause an approximation of the limb buds behind, then the two buds would approximate by what in adult life are their outer borders; that is to say, by the parts which the little toes denote. This may, perhaps, be more clearly understood if the two hands be placed on either side of the pelvis, with the palms against the body and the thumbs upwards. A defect in the pelvis would cause the approximation of the edges of the little fingers, greater or less in degree, according to the amount of the defect.

But a mere approximation of the parts is not in itself sufficient to cause coalescence, and it is not sufficient to cause those actual defects in the structures which we have seen to exist. It seems to us that the state of matters implies that some injury at this early period has not only removed or destroyed some of the parts, but has, as in the case of raw surfaces in the adult, induced a tendency to coalesce. It looks as if a bit of the floor of the pelvis had been, as it were, pinched out, so that the germinating tissue was broken up, the epiblastic or cuticular layer being removed, so that it acquired a tendency to adhere

to the structures opposed to it. The injury to the tissue in some cases involves the limb buds themselves, as distinguished from the pelvis, so that there may be great destruction of them, and a mere aborted extremity formed, as in *sympus apus*. Or the limb buds may be left nearly unaffected, and their whole parts, at least so far as the skeleton is concerned, may be perfectly developed, as in some cases of *sympus dipus*. All this implies that the lesion occurs when there is little more than the limb girdle and the first buds present. The formation of the limb girdle, which afterwards forms the pelvis, occurs about the same time as that of the buds, namely, about the fourth week of utero-gestation.

That the lesion is something in the nature of an injury is evidenced further by the condition of the intestine, and by the occasional further defects in the urino-genital organs, not merely the interruption of passages, but such circumstances as the absence of the external genitalia, and various defects of the kidneys.

It may be further remarked that the injury is essentially one of the middle line, producing a malformation which is virtually symmetrical, although not in every case absolutely so. It is curious that in our case, for instance, there is a doubling of the lower end of the femur and upper end of the tibia; but, on the one side, the greater part of the foreleg has, as it were, been picked out.

The question next occurs as to the nature and mode of production of such an injury at an early period of fœtal life. Cruveilhier¹ suggested that the action of lateral pressure on the posterior parts of the embryo might produce it. But it seems to us that lateral pressure could scarcely produce such a definite lesion, limited in its primary seat to the middle line; nor would it explain what to us is so obvious as the principal fact, the removal or suppression of certain structures. Dareste² agrees with Cruveilhier that the cause is to be found in pressure, but does not see how external pressure could cause the singular rotation of the limbs, which is so characteristic. He claims that he has found the true explanation, in an arrest in the development of the posterior part of the amnion, which he calls the *Capuchon caudal*. By this arrest the capuchon is not expanded below the embryo, but rests on the latter instead of separating from it. In consequence of the constriction by this part of the amnion, the limb buds are turned backwards, and come to be placed towards the dorsum of the embryo, approximating by their external aspects, which have now become internal. This looks more like a correct explanation, but it does not by any means account for the defect in the pelvis, which seems to us to be a primary fault. Nor does it explain, as Dareste is careful to point out, the defects in the urino-genital and intestinal tracts, which are such constant concomitants. We regard it as probable that defect of

¹ Cruveilhier, "Traité d'Anat. pathol.," i. 311.

² Dareste, "La production artificielle des Monstruosités," 2nd ed., 1891, p. 420.

the amnion, either by way of simple non-expansion of the caudal end or abnormal adhesion, so as to interfere with the growing structures, is the cause of the malformation.

The peculiar position of the coalesced limb has been frequently referred to. In this connection it is to be remembered that, in the process of development and expansion of the foetus, both the upper and the lower limbs undergo a process of rotation. The lower limbs appear as two lateral buds, which are applied to the sides of the body. They are so applied that the plantar aspects, or what will become the plantar surfaces, face inwards or even slightly forwards, and are in contact with the body. The primitive toes are spread out, and are counted from above downward, the great toe being the first and uppermost. In the normal process of development, the limb-buds suffer a rotation, so that the plantar surfaces ultimately face downwards and the great toes inwards, although at the time of birth, and even for a time after it, the soles of the feet frequently look inwards, and the great toes upwards.

The lesion under consideration will altogether prevent this rotation. The parts which become the outer borders of the limbs, by the defect in the middle line, are brought into contact and adhere. The coalesced limb is thus fixed, with the outer borders adhering in the middle line, and the limb grows out with the plantar aspect facing forwards, the great toes outwards, and the little toes, more or less coalesced or suppressed, internal.

THE PATHOLOGY OF THE VERMIFORM APPENDIX.¹

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INTRODUCTION.

THE following paper is based upon a research undertaken with the object of elucidating some of the complex problems connected with the surgery of the vermiform appendix. This investigation comprised:—

A careful examination of the appendix in 100 cases; such investigation embracing the anatomy of the cæcum, the peri-cæcal and retro-cæcal fossæ, the dimensions of the appendix, the presence or absence of the various cæcal folds, and other anatomical details. These results will be published *in extenso* elsewhere.² Here it will suffice to say that I believe that the peri-cæcal and retro-cæcal fossæ have a most important bearing upon the pathology of the appendix.

Secondly, an inquiry was instituted into the histology of the appendix throughout the vertebrate kingdom. The results of this inquiry prove that the morphological analogue of the human appendix is a mass of lymphoid tissue, usually situated at the apex of the cæcum.

Thirdly, a series of operative experiments were performed upon rabbits; the results almost conclusively proving that the primary function of the appendix is the production and exudation of leucocytes; and, further, that the functions of the appendix are to be looked for in these—its microscopic constituents—rather than its fluid and unformed elements, as has hitherto been done by those who have not altogether denied that the appendix has a function.

Such investigations, naturally enough, cleared up several important points connected with the pathology of the organ under discussion. It is with these that this paper is more especially concerned.

¹ Part of a Graduation Thesis presented for the degree of M.D. in the University of Edinburgh, and for which the Gunning Prize in Surgery was awarded.

² *Anat. Anz.*, Jena, 1895, bd. x. s. 401.

PATHOLOGICAL CONDITIONS OF THE VERMIFORM APPENDIX.

1. Congenital anomalies and malformations.
2. Abnormalities in position.
3. Atrophy of the appendix.
4. Cystic dilatation of the appendix.
5. Foreign bodies and calculi.
6. Inflammatory lesions—
 (a) Simple. (b) Specific.
7. New growths.

1. *Congenital Anomalies and Malformations.*

This division is of comparatively little importance, and its lesions are rare. Their rarity may, however, have been thrown into greater prominence by the fact that the appendicular inflammatory lesions have usurped public attention to the exclusion of almost all the others.

Here may be included persistence during adult life of the foetal or embryonic type of cæcum and appendix. Instances of this have been met with in the author's anatomical series, and also in the writings of Treves (1), Struthers (2), Bennet and Rolleston (3), and others.

Malformations of the appendix are too rare to call for any mention.

2. *Abnormalities in the Position of the Appendix.*

Positional abnormalities of the appendix fall naturally into two groups; under the first and smaller group may be included those abnormalities in position, the result of a non-descended cæcum. Several such instances are recorded, and their importance is to be sought in the possibility of an appendicitis occurring in an appendix so situated, when the results might be puzzling to both the physician and the surgeon. I have met with one such case in a boy aged 8 years.

The second and larger group of cases comprise the various herniæ of the appendix. These may be subdivided into external and internal herniæ.

(A) *External herniæ.*—By an external hernia of the vermiform appendix is meant one occurring in any ordinary surgical external hernia, and produced by the same causes as an ordinary hernia.

This variety is of considerable importance, though perhaps not quite so interesting as the internal variety, because the latter is more especially concerned in the etiology of the inflammatory lesions.

The vermiform appendix may form part of the contents of—

A right-sided inguinal sac.

A right-sided femoral sac.

Some rarer forms of hernial sac.

In point of frequency the order is that here given.

(1) *A right-sided inguinal hernial sac.*—For the production of this, the most frequent variety of an appendicular external hernia, it is absolutely essential to have either a long or a very freely movable appendix,

with or without a freely movable cæcum. Further, the extent to which the appendix will enter the hernial sac must also depend upon these factors.

The condition is more frequent in children than in adults, as far as can be judged from recorded cases.

Once in a hernial sac, the appendix may form adhesions, become inflamed, or may ulcerate.

Cases of appendicular hernia in a right-sided inguinal hernial sac appear to be fairly common, and have been recorded by Kelynack (⁴), Lockwood (⁵), Habershon (⁶), Le Bec (⁷), and many others. I have met with no cases myself.

(2) *A right-sided femoral hernial sac*.—An appendix so herniated may undergo the same changes as the inguinal or first variety.

Interesting cases have been recorded by Annandale (⁸), Swasey (⁹), Hall (¹⁰), and several others.

(3) *Some rarer forms of hernial sac*.—The cases of herniation of the appendix under this head are exceedingly few. Geissler records a case in a patient æt. 70, where the appendix was found in the sac of a left-sided inguinal hernia.

The importance of these external herniæ of the appendix is most obvious in connection with the inflammatory lesions of that organ; as in a case quoted by Thurmann, where an appendix, herniating in the manner described, produced a scrotal appendicitis.

(B) *Internal herniæ*.—Internal herniæ of the vermiform appendix have, until within recent years, been entirely overlooked. This, of course, resulted from the lack of a due appreciation of the importance of the peri-cæcal and retro-cæcal fossæ; consequently, in the author's anatomical researches, a considerable amount of attention was paid to these fossæ.

The importance to the surgeon of herniæ of the appendix into these fossæ can hardly be overestimated.

In the first place, such a hernia of an appendix may in itself be the cause of an appendicitis. That a hernia of the appendix into a retro-cæcal fossa may predispose to an attack of appendicitis I have not the slightest doubt; whether a peri-cæcal hernia may act in a similar manner is not quite so clear. Talamon (¹¹) raises this point when he remarks: "It is only right to inquire if, in certain cases, the appendix could not become involved and confined in this fossa of Treves (that is, the ileo-cæcal fossa—one of the peri-cæcal division), and thus, if such an anatomical arrangement might not become a cause of appendicitis, or rather of appendicular colic. It does not, however, appear that such an abnormal distribution of the diverticulum has ever been noticed in the course of the numerous researches undertaken during the last few years in order to ascertain the exact relations of the appendix." The question here raised by Talamon is pertinent enough; his answer to his own question is, however, erroneous, as I showed in a footnote to the above paragraph in the English translation of his work.

In the second place, internal herniation of the appendix is important to the surgeon because, given an appendicitis with the appendix so situated, the success of any operation may be imperilled, or indeed absolutely negatived thereby. It is, therefore, of paramount importance for a surgeon to be acquainted with the frequency and situation of such herniæ of the vermiform appendix.

(1) *Hernia of the appendix into the peri-cæcal fossæ (ileo-colic and ileo-cæcal).*—Hernia of the vermiform appendix into the ileo-colic fossa rarely occurs, for the simple reason that the fossa is far too small to allow of such herniation. In my own anatomical series, such an internal hernia was never found, and in an overwhelming majority of cases the size of the fossa absolutely forbade the occurrence of such a condition.

Further, I have nowhere been able to find any recorded example of a hernia of the vermiform appendix into the ileo-colic fossa.

Hernia of the appendix into the ileo-colic fossa probably, then, never occurs and never produces an appendicitis.

So recent a writer as Jonnesco (¹²), in a work specially devoted to internal abdominal herniæ generally, remarks that "ileo-cæcal hernia of the appendix is rare." In fact, he only mentions one case—that of Snow (¹³). He has never met with such an example.

(2) *Herniæ of the appendix into the retro-cæcal or secondary cæcal fossæ.*—Numerous examples of such herniation are on record. Without going into details, suffice it to say that cases will be found described by Lockwood and Rolleston (¹⁴), and by C. B. Lockwood, both in the *Transactions of the Pathological Society of London* (¹⁵), and in his *Hunterian Lectures* of 1889 (¹⁶), also by Macalister (¹⁶), Dunn (¹⁷), and most recently by Jonnesco (¹²).

Several examples of retro-peritoneal herniation of the vermiform appendix were also found in my own series of cases.

The importance of this herniation is chiefly as a predisposing cause of appendicitis.

Summary of hernia of the appendix.—Hernia of the appendix into the retro-cæcal fossæ is the most frequent form of appendicular herniation.

Hernia of the vermiform appendix in ordinary external abdominal herniæ is of comparatively frequent occurrence.

Hernia of the appendix into the peri-cæcal fossæ is rare.

The importance of hernia of the appendix, whether external or internal, is chiefly as a predisposing cause of appendicitis, and to a less extent as masking the symptoms of that disease, and so obscuring the diagnosis.

3. *Atrophy of the Appendix.*

That atrophy of the appendix occurs has, I think, been already fully proved by Ribbert (²⁷). Whether such atrophy is physiological or pathological is doubtful. My observations confirm those of Ribbert; I regard atrophy as a physiological process, occurring in late middle, and old age.

4. *Cystic Dilatation of the Vermiform Appendix.*

Accounts of such cystic dilatation of the appendix will be found in the writings of Féré (¹⁸), who terms the condition a "mucocele"; of Gruber (¹⁹), Guttman (²⁰), Shoemaker (²¹), Weir (²²), and others. Other references to records of such cases will also be found in the recently published work of Kelynack (²³).

Shoemaker's case is precisely analogous to a case of the writer's. In both, the condition was only discovered post-mortem, and in both gave rise to no symptoms during life.

That this dilatation of the vermiform appendix results from retained secretion has been proved by my experiments upon rabbits, where the appendix always became dilated on the distal side of a ligature applied round the base of the organ.

The most important question under this head is that of the relations of the condition to appendicitis. Is there any relation between the two conditions? Are they cause and effect?

Briefly, I may state that I believe cystic dilatation of the appendix bears a most important relationship to the etiology of appendicitis.

5. *Foreign Bodies and Calculi.*

The literature of the subject reveals a most extraordinary collection of substances which are stated to have been found within the cavity of the appendix.

Thus fruit-seeds, cherry-stones, hairs, bristles, shot, pins, gall-stones, lumbricoids, pills, teeth, peanuts, shells, and bones, have at all times been recorded as having been found within the appendix.

Without challenging the good faith of the observers who have recorded the above examples, I venture to assert that many of them are inaccurate. From their similarity in size, shape, and appearance, so-called faecal concretions have frequently been mistaken for foreign bodies. It may be said, in fact, that the occurrence of foreign bodies, properly so-called, within the cavity of the appendix, is exceedingly rare, and probably much more so than has been usually believed; in proof of this statement, there are the following facts:—That in my series of anatomical cases, in no single instance did I find a foreign body of any description. Concretions were found in several instances, but these are not included under the present term.

A close investigation of the appendix in that series proved that the anatomical position of the appendix in very many cases renders it a physical improbability that any foreign body, particularly one the size of a cherry-stone, or even a shot, could enter that organ. When we consider that, in many cases, the appendix runs in a diametrically opposite direction to the cæcum; that the orifice of the appendix is extremely small; that the nature of the junction is oblique, as Struthers

has pointed out; and, lastly, that experimentally it is often very difficult to make a fluid, such as water, enter the appendix from the cæcum, the physical improbability of the entrance of cherry-stones and so forth at all frequently into the appendix becomes obvious.

Another striking point in favour of such a statement, is the fact that most of the recorded examples of such extraordinary foreign bodies within the appendix were published before attention had been prominently turned towards that organ. Thus pins, bristles, lumbrici, and so forth, were rife within the appendix about the period 1820 to 1870. But notwithstanding that foreign bodies are rarely met with in the appendix, concretions are by no means uncommon; a statement which requires no further proof. To-day, indeed, no one will dream of contesting it. It has already passed from the quicksands of debate to the solid foundation of fact.

The term "fæcal concretion" should give way to that of "*appendicular calculus*," as the former is misleading.

All the so-called "fæcal concretions" I could obtain from undoubted cases of appendicitis (for many of the opportunities so afforded me I have to express my thanks to Mr. Charles W. Cathcart, F.R.C.S.) were submitted to a careful chemical examination, with the result that in no single instance were the concretions found to consist at all largely of fæces. They were composed principally of inspissated mucus and various salts, and were merely stained with fæcal colouring matter.

These results are further confirmed by the writings and public statements of Knipe (²⁴), Pepper, Smith (²⁵), and other authors; all bearing out my point, that the so-called fæcal concretion is rarely composed to any appreciable extent of fæces. Therefore the term "fæcal concretion" should give place to "*appendicular calculus*."

Appendicular calculi are formed within the appendix. Talamon's statement, that they are driven from the cæcum into the appendix, is entirely erroneous.

In support of this assertion, as has just been stated, the calculi are rarely composed of fæces alone. If they were forced into the appendix from the cæcum, as Talamon asserts, it is obvious that they must of necessity be composed of fæces, whilst, further, they would be frequently found in the cæcum post-mortem; and, as a matter of fact, the presence of such calculi within the cæcum is, to say the least, extremely doubtful.

Then, again, the physical improbability of the passage of such substances from cæcum to appendix has already been demonstrated.

On the other hand, the formation of calculi within the appendix is supported by experiment.

A most striking instance of this may be found in some operative experiments performed by L. Hermann (²⁶). These observations are so important, and bring out so strikingly the practical significance of my own experiments, that a brief summary of Hermann's paper is here given.

Hermann's operations consisted in resecting a portion of intestine, suturing the ends together, and leaving the ring so produced in the abdominal cavity. The extremities of the gut, after removal of such a ring, were of course sutured together in order to preserve a general intestinal passage. Before closing the resected ring, it was carefully washed out, so that any subsequently-produced contents must of necessity be the result of intestinal secretion. What was the result?

The circle of intestine was filled with a brownish exudation, jelly-like or clotted, and contained enormous numbers of bacteria and leucocytes, fat granules, and so forth, but no epithelium or traces of food.

In one of his most successful cases, a bitch, operated upon on May 6th and killed on the 22nd May, the ring felt to the touch like a sausage, and contained a solid greenish-grey mass, exactly like *fæces*, but with no fluid. The mass was something like icteric *fæces*, but was more homogeneous than *fæces* usually are. This mass weighed 60 grms., and on standing changed from green to brown. It was found to contain bacteria, leucocytes, mucin, etc., and could only have been produced, says Hermann, by internal secretion and inspissation. He next goes on to say that *fæces* may possibly be very largely due to intestinal secretion rather than to food, in proof of which he adduces the fact that starving people pass *fæces*. The pertinent question is then asked, If the pultaceous mass which always fills the *cæcum* of a rabbit, may not be largely due to *cæcal* secretion, although it does consist to a slight extent of food particles?

In an experiment of my own, I succeeded in producing such a pultaceous mass within the appendix as a result of ligature, thus confirming these experiments of Hermann. The conclusion seems indubitable that the calculi found in appendicitis are the result of the appendicular secretion, and are solely formed within the cavity of that organ.

Finally, another strong argument against Talamon's assertion is to be found in the work of Ribbert (²⁷). In the course of some extremely interesting remarks, this author states that only the smaller stones consist exclusively of *fæces*. As soon as they attain a larger diameter than the normal lumen of the appendix, and thus produce a dilatation of the process, these concretions will be found to consist centrally of *fæces* covered by a coat of mucus. He proceeds to say that if we harden an appendicular process, containing one of the stones, and make sections through both, and stain with Weigert's fibrin method, the mucin masses will be stained blue, and it can then be demonstrated that the mucus of the outer laminae of the stones is directly continuous with the mucus of the ducts of the glands. The concretions then grow in size by a deposition upon them of mucus.

Enough has been said to prove the accuracy of my statement, that *fæcal* concretions are formed within the appendix and contain little or no *fæces*, and would therefore be better termed, from an analogy with renal and hepatic calculi, appendicular calculi.

Lastly, I find that appendicular calculi are equally common in both sexes, and are most frequently met with between the ages of 20 and 30—about that very period when the appendix shows its greatest functional activity and when appendicitis most frequently occurs.

The results of Ribbert's work here support my own investigations.

6. *Inflammatory Lesions of the Appendix.*

It is not intended here to do more than merely classify the inflammatory lesions of the organ, because where such an enormous flood of literature already exists it is best rather to simplify than to add to the existing complexity.

The inflammatory lesions of the appendix may then be classified as follows:—

(A) *Simple inflammatory lesions—*

1. Non-perforative or "medical" appendicitis. This includes all the mild forms of appendicular colic, the majority of which may be treated by the physician.

2. Perforative or surgical appendicitis. This includes all the graver forms of the disease, surgical interference being absolutely essential.

3. Relapsing appendicitis. Also a surgical affection.

(B) *Specific inflammatory lesions—*

1. Tubercular appendicitis.

2. Typhoid appendicitis (rare).

3. Actinomycotic appendicitis (very rare). Of this there is only one recorded instance.

Although at the time of classifying the inflammatory lesions of the appendix I was not aware that any other author had adopted a similar one, I find that Kelynack's classification is practically identical. Kelynack does not, however, differentiate between the medical and surgical appendicitis, as is done above.

7. *New Growths of the Appendix.*

Primary new growths within the appendix are so rare as to be practically unknown. I have never yet met with any example of simple or malignant tumour formation within the appendix.

Several cases of carcinoma of the appendix have been recorded, but the details are generally obscure. Thus in Draper's (28) case, although entitled "Colloid Cancer of the Vermiform Appendix," an investigation into the details makes it almost certain that the case was primarily one of carcinoma of the ileo-cæcal valve, involving the appendix.

Other so-called cases of carcinoma of the vermiform appendix are similar to Draper's.

In the entire literature of the subject the only examples of new growths I can find are carcinoma and sarcoma, and both were secondary.

THE ETIOLOGY OF APPENDICITIS.

The causes of appendicitis may be divided into—

1. *Predisposing.*

Anatomical location of appendix.
Indigestion.
Constitutional functional disturbances.
Occasional.

2. *Exciting.*

Appendicular calculi.
Micro-organisms, especially the *Bacterium coli commune*.
Tubercle.

Anatomical location of appendix.—This I regard as of the highest importance. Any position of the appendix which tends to interfere with the free flow of matters from appendix to cæcum must be looked upon as an important predisposing cause of appendicitis.

Such positions are principally lodgment of the appendix within one of the cæcal fossæ, and more frequently within the retro-cæcal than the pericæcal set.

Other predisposing positions are such as tend to produce any decided kinking in the organ. These are not so frequent as the retro-cæcal situations. How these predisposing positions act will be shown immediately.

Indigestion.—Under this head Talamon (¹¹) says: "Indigestion, or at least the ingestion of indigestible food, is so frequently pointed to as the origin of the symptoms that it is impossible not to look upon this condition as a frequent determining cause . . . The passage through the intestine of foods taken in excess, badly digested or badly tolerated, such as cabbages, mushrooms, carrots, turnips, game which is too high, etc., provoke abnormal movements of the intestinal canal, and thus determine the engagement of the fæcal calculus in the appendix."

That indigestion is an important predisposing cause of appendicitis I am fully convinced. That it acts as Talamon avers I totally deny. No amount of abnormal intestinal movement could, in the majority of instances, force any body from the cæcum to the appendix; and in the second place, I have already endeavoured to prove, and I think successfully so, that such calculi are not formed within the cæcum, but within the appendix, and hence Talamon's explanations of indigestion as a predisposing cause cannot be admitted.

In my opinion, indigestion, as a factor in the production of an appendicitis, is to be looked upon as an adjuvant to the first cause—
anatomical location of the appendix.

Indigestion alone will not produce an appendicitis, or at all events very rarely. In a person whose appendix is retro-cæcally situated, or is situated, in short, in any position which tends to prevent free flow of

matters from appendix to cæcum, the occurrence of indigestion becomes a serious matter, and almost certainly induces an attack of appendicitis. What happens?

In such a case, the appendix, from its anatomical situation (let us assume), has at any time the greatest difficulty in driving its secretion on into the cæcum. Such a state of affairs leads naturally to the formation of appendicular calculi, as has already been shown. These if small will produce no result. An attack of indigestion supervenes. In consequence of increased peristalsis the appendix is irritated, its secretion increased, violent efforts are made to expel the calculi, and the patient has an attack of appendicular colic.

In support of such a theory is the fact, that many persons who have had several attacks of appendicular colic have, by paying strict attention to their diet and to the daily evacuation of their bowels, ceased to have such attacks. Several such instances are well known to the author.

Indigestion is, then, an important predisposing cause of appendicitis, but certainly not in the manner indicated by Talamon. So important, indeed, is indigestion that when associated with an unfavourable position of the appendix it may be regarded as an exciting cause, rather than as merely a predisposing one.

Constitutional functional disturbances.—The point that I here desire to especially emphasise is the fact that appendicitis is not always due to local causes alone, but may be the localised manifestation of a general condition.

The fact, although obvious enough in itself, has not yet been sufficiently recognised.

The vermiform appendix is largely composed of lymphoid tissue; a long research undertaken by the author upon the comparative histology of the appendix having proved that the morphological analogue of the human appendix is found almost throughout the entire vertebrate kingdom in the shape of lymphoid tissue at the apex of the cæcum. Therefore it may be said that lymphoid tissue is the characteristic component of the vermiform appendix as found in man.

Lymphoid tissue, it is well known, is characterised by a tendency to inflammation, particularly in the case of young people. An everyday example of this is to be found in inflammations of the tonsils; many of which occur without any obvious cause.

In the same way the appendix, largely composed of lymphoid elements, may undergo inflammations without obvious causes or from constitutional and functional disturbance.

Appendicitis then may be, and doubtless often is, the localised result of some general and functional disturbance.

The proof of this is to be sought in the tonsillar analogy to which reference has just been made.

Kelynack writes as follows: "The reasons, I believe, depend upon the well-recognised tendency for lymphoid tissue to become the seat of

inflammatory processes in early life. The appendix is usually very rich in such lymphoid structure, and it would seem that in early life it is much more liable to acute and rapidly extending inflammation than is the case in adult or advanced life."

Lastly, Sutherland (²⁹), in a paper published in 1892, has endeavoured to emphasise this relation of the appendix to general functional disturbance by showing that there exists in children a close relationship between that organ and the uric acid diathesis.

Occasional causes.—By these are meant such rare and ill-defined causes as traumatism, cold, and so forth, which do not call for any further mention. Here also may be included those other occasional causes which I regard as of secondary importance only, namely, age, sex, diet, antecedent illnesses, etc.

2. *Exciting Causes of Appendicitis.*

Appendicular calculi are generally admitted to be the most frequent cause of appendicitis. The disputed point is their mode of action.

According to Talamon the calculi are formed within the cæcum and thence driven into the appendix, so producing appendicular colic just as biliary colic is produced by the passage of a gall-stone. Further, the calculi, according to Talamon, may lower the vitality of the appendix, and so perforation may result from the presence of bacteria, producing ulceration.

As the calculi are not formed within the cæcum, and are therefore not driven into the appendix, such a theory falls to the ground.

The explanation of the occurrence of those appendicitis becomes simplicity itself, if the process be reversed, for I maintain that any obstruction to the flow of matters from appendix to cæcum may eventually result in the formation of calculi within the appendix. These, if small, produce no symptoms. If larger, their passage from appendix to cæcum gives rise to appendicular colic. If still larger, or if associated with some obstruction from kinking of the appendix, or so forth, then the calculi become impacted at the seat of obstruction; the appendix, an inelastic organ, dilates behind the obstruction and becomes first the seat of fermentation, and secondly of inflammation as the result of that fermentation; with gangrene or perforation dependent upon the intensity of the inflammation.

This view harmonises with the facts of the case, and efficiently explains the following circumstances:—

The presence, post-mortem, within the appendix of small fæcal concretions, the appendix never having been the seat of disease during life.

Such a theory also explains the occurrence of a gangrenous appendix, the calculi being found lying loosely at the apex of the diseased organ. This actually occurred in one of my own cases. The explanation is simple. The calculi were formed within the appendix. Their passage

along that organ produced the well-known signs of appendicular colic. The calculi then became impacted at some portion of the appendicular canal, and then the organ slowly dilated behind the obstruction. Gangrene resulted from the intensity of the inflammation, and the calculi now dropped back to the apex of the organ, as, in consequence of the dilatation of the organ, they were no longer impacted.

The occurrence of perforation is also readily explicable upon this theory.

The calculi in their passage along the appendix damage the mucous membrane; micro-organisms invade the damaged area, and so perforation results, either at the present seat of the impacted calculus, or at its former seat. This is the point which Talamon says the theory of intra-appendicularly-formed concretions fails to explain. It, however, seems sufficiently obvious.

The occasional absence of appendicular calculi is also explained on this assumption, although Talamon, with his theory, finds it extremely difficult to explain away such occasional absence thus:—

The appendicitis may be due to one of the non-calicular causes, as has already been explained.

The appendicitis may be due to some dilatation of the organ behind a temporary stricture, however produced. In this case gangrene, or turgescence of the organ, may result without the appendix ever having contained a calculus at all.

The calculus may have passed on into the cæcum, or may have disappeared through a perforation into the peritoneal cavity.

Micro-organisms.—That micro-organisms play a most important part in the etiology of appendicitis is a fact too well established to be contested.

Naturally, there are a number of interesting details in the bacteriology of the appendix, but they are beyond the scope of the present paper.

Tubercle.—That tubercle will eventually be found to play an important part in the production of appendicitis seems probable. The anatomical facts certainly all point to such an occurrence.

The lymphoid character of the appendix has been referred to more than once in this paper; the statement must here be accepted that such is the case; the proofs will be supplied elsewhere. The tendency of tubercle to attack such tissue is too well known to need more than passing mention; therefore, the conclusions seem almost inevitable, that an examination of the appendix in cases of tuberculosis, will prove that organ to be affected with tubercle; secondly, that if an appendix which has been removed for appendicitis, be examined for tubercle bacilli, that organism will be present in many cases; while, lastly, it may be laid down as a clinical fact that a closer examination will show that appendicitis is relatively by no means unfrequent amongst tubercular subjects.

That these conclusions are not unsupported may be shown by the following facts.

I have met with one human subject in whom the appendix was the seat of tubercular ulceration.

The case was one of hip-joint disease, treated by excision; phthisis supervened; from which the patient died, the post-mortem yielding the above result. It may be argued that one case does not prove much, but then the case only came to my notice by chance. I have not as yet systematically examined appendices for tubercle, although a careful study of the anatomy and histology of the organ has led me to believe that tubercular affections will eventually be found to be of by no means unfrequent occurrence.

I have also met with a rabbit, the subject of tubercle, where the appendix was similarly affected.

The lymphoid tissue at the apex of the cæcum, in a specimen I examined of the European beaver, was also tubercular.

Lastly, various authors support this statement. Thus Habershon (*), states that in "phthisis it is very common to find ulceration in the appendix cæci, from the degeneration of tubercle."

Walsh, Ziegler, Kelynack, and others, also support such a view.

SUMMARY OF THE ETIOLOGY OF APPENDICITIS.

1. *Non-Calicular Varieties.*

Appendicitis may, first, be the localised result of a general functional disturbance, due either to the uric acid diathesis, or to those obscure causes which tend to produce inflammation of lymphoid tissue in young people generally.

Secondly, the disease may be, and often doubtless is, a manifestation of tubercle. Careful examination of future cases will probably show that tubercle is a more frequent factor in the etiology of the affection than is at present imagined.

Appendicitis, and particularly the mild forms of the affection, are doubtless often due to causes which induce a slight obstruction in the appendicular canal, thereby interfering with the free flow of materials from appendix to cæcum. The increased efforts of the appendix to overcome such obstruction produce an attack of appendicular colic. Less frequently cystic dilatation of the appendix results from the same cause—obstruction, in which case the dilatation of the organ is chronic, and therefore associated either with very mild symptoms of appendicitis, with relapsing appendicitis, or with no symptoms.

Such obstruction may be temporary and evanescent, as, *e.g.*, from kinking of the appendix, produced by any mechanical or intra-abdominal disturbance; by pressure upon the appendix from without, as from an overloaded gut or tumour formation, or lastly, and more permanently, by some of the abnormalities in position assumed by the appendix.

These three varieties of appendicitis—general functional appendicitis,

tubercular appendicitis, and simple obstructive appendicitis, may be termed "non-calicular" varieties, inasmuch as they are not calicular in origin, and calculi are not usually found in the cavity of the appendix in such cases.

The non-calicular varieties of appendicitis may however undergo gangrene or perforation just as may the calicular, but not so frequently. The mechanism of gangrene or perforation in such cases is then as follows:—

The appendix dilates behind the obstruction wherever situated, the retained secretion behind that obstruction undergoes fermentation from the presence of the micro-organisms which the appendix is known to contain; a septic or infective inflammation is thus induced; gangrene and perforation depending upon the intensity of that inflammation.

The non-calicular varieties are probably not so dangerous as the calicular varieties, and doubtless include most of the mild or medical forms of the disease.

2. Calicular Varieties of Appendicitis.

These include the majority of the graver forms of the disease and also the majority of those forms which have been termed "surgical."

Their exciting cause is the same in every instance, namely, calculi. The variety may also be made to include those rare forms due to actual foreign bodies introduced from without.

The calculi are produced within the appendix, and probably as the result of some obstruction in the lumen of the appendix. Just as in the case of calculi, in other parts of the body, we are not yet in a position to appreciate their exact mode of origin.

Their passage along the appendix usually gives rise to appendicular colic; indigestion acting as a powerful predisposing cause in the manner already described.

The mechanism of gangrene and perforation, as produced by these calculi, has already been described.

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A CONTRIBUTION TO THE BIOLOGY OF THE RINGWORM ORGANISM.

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IT is not my intention in this paper to discuss the biology of the ringworm organism in its various phases, but simply to draw attention to some points concerning the production of ferments by the Trichophyton. The object of the research was to determine whether the ringworm organism in the course of its growth in various soils outside the body produces ferments, and, if so, what their nature is.

The nature of the ferments produced by the bacteria has been investigated by Bitter, Fermi, Lauder Brunton, the writer, and others. It has been found that those bacteria which liquefy gelatine do so by means of a soluble ferment. The action of this ferment on gelatine can be demonstrated apart from the cells that produce it. Thus, if one adds the liquefied gelatine alone to fresh gelatine, a liquefaction takes place. In a similar way it has been proved that the bacteria produce diastatic and milk-curdling ferments.

The present writer's investigations in this direction had hitherto been confined to the bacteria. It was with a view of extending these investigations to the group of the moulds, that the ordinary *Trichophyton tonsurans* was selected. Could one demonstrate the production of ferments by the moulds, as had already been done in the case of the bacteria?

The pure cultures of the trichophyton were obtained from Dr. Kral of Prague. They corresponded in their morphology and growth with the trichophyton associated with ringworm in the scalp in children. The morphology of the Trichophyton is not touched upon in this paper, a subject which is being so carefully worked out by Dr. Sabouraud and others.

Dr. Sabouraud believes that the Trichophyton organisms consist of different species, but that they all belong to the genus *Botrytis*. He describes two varieties of Trichophyton in ringworm, namely, the *T. microsporon* (in 60 per cent.), and the *T. megalosporon* (in 40 per cent.).

Rosenbach describes seven groups of Trichophyton isolated by him.

The full details will be found in the works these writers have recently published.¹

One thing at anyrate is certain, that the constitution of the soil on which they are grown exercises a considerable influence on their manner of growth. Their appearance, also, varies according to the temperature at which they are grown. In studying the morphology of the Trichophyton group, it is most important that the cultures be kept at a uniform temperature.

The first series of experiments were made to determine whether the *T. tonsurans* produces a peptonising enzyme.

Tubes of 10 per cent. gelatine were inoculated with the ringworm organism, and placed in an incubator at 20° C. The trichophyton in the course of its growth completely liquefied the gelatine. When liquefaction was complete, a few drops of the liquefied gelatine were transferred to the surface of sloping gelatine. The tubes were then placed at 20° C., a temperature at which the gelatine remained solid. The result was a distinct dimpling of the surface of the gelatine at those points where the liquid gelatine rested upon it. As the liquefaction advanced the gelatine became distinctly furrowed. The liquefied gelatine collected at the lower part of the tube. It remained quite clear, and showed no traces of growth. The gelatine from the ringworm culture had therefore liquefied fresh gelatine.

In a second series of experiments the sterile gelatine was first liquefied, and a few drops from a gelatine culture of ringworm were well mixed with it. The gelatine was then allowed to resolidify, and kept as before at 20° C.

The following day the tubes were removed from the incubator, and cooled down under the tap. It was found that in the short space of 15–17 hours, two-thirds of the gelatine had become liquid; only the lower third in the tube regelatinised on cooling. The liquefied gelatine remained clear and free from any visible growth. Here, again, there was evidence of a marked dissolving action on gelatine, independent of the growth of the organism itself.

A further series of experiments were made in the following manner:—

The liquefied gelatine from a ringworm culture was poured on the surface of sterilised gelatine in a test tube. A mark was made on the tube to indicate the line of demarcation between the two layers of gelatine. The tubes so prepared were again kept at 20° C. On examination, after 18 hours, it was found that 5 mm. of the sterile gelatine had been liquefied, without any trace of growth of organisms. After 6 days the gelatine was liquefied to the depth of 11 mm. These experiments were repeated, with the addition of chloroform to the gelatine, and the results were the same—liquefaction of the

¹ "Les Trichophyties Humaines," par Dr. Sabouraud. Paris, 1894; "Ueber die tieferen eiternden Schimmelerkrankungen der Haut," von Dr. Rosenbach. Wiesbaden, 1894.

gelatine. These preliminary experiments gave evidence of the presence of an active enzyme in the ringworm cultures, which was capable of liquefying gelatine at a comparatively low temperature.

The experiments were continued in the following fashion. A tube of fresh gelatine was inoculated with the trichophyton. A second tube was inoculated with a few drops of gelatine, which had previously been liquefied by the ringworm organism. We had therefore a tube sown with ringworm alone, and one containing only the liquefied gelatine from a culture. The tubes were placed at blood heat, and examined on the following day. The tube inoculated directly with ringworm showed no growth, and the gelatine resolidified completely on being cooled down. The tube of gelatine to which the "enzyme gelatine" had been added was quite liquid, and remained so at room temperature. This experiment showed that the gelatine, which had been liquefied by the growth of the trichophyton, contained a soluble ferment, capable of liquefying gelatine independently of the cells that produced it. The amount present in a few drops was sufficient to completely liquefy about 10 c.c. of fresh gelatine. The gelatine, in which a growth of the trichophyton had barely started, did not yet contain this active ferment.

The experiments were continued at blood heat. A number of tubes of 10 per cent. gelatine were inoculated with ringworm. A further series were inoculated with a few drops of liquefied gelatine from a ringworm culture. The tubes were examined the next day. The tubes inoculated with the trichophyton showed neither a growth nor liquefaction of the gelatine. The tubes to which the "enzyme gelatine" had been added were completely liquefied. It was found that this complete liquefaction of the gelatine took place in 17-18 hours. The ferment acted more quickly and powerfully at blood heat than at 20° C. In the case of the tubes which had been kept at 20° C. the liquefaction of the gelatine was partial, whilst at blood heat the liquefaction was complete in the same space of time.

It will be seen that the peptonising enzyme secreted by this trichophyton is of a very active nature—a 10 per cent. solution of gelatine being liquefied within 18 hours. To render the experiments more conclusive, they were repeated with the addition of an antiseptic substance to the gelatine. A few drops of the oil of wintergreen were added to the tubes before inoculation. The oil retarded any growth of organisms, but did not interfere with the action of the ferment. The results were the same as detailed above, namely, complete liquefaction of the gelatine at blood heat by the enzyme. The liquefaction, however, is so rapid, that the addition of an antiseptic is hardly a necessary precaution.

The ferment action of the ringworm cultures was next tested at a temperature of 30° C. Five series of tubes were prepared in the following manner:—

1. Gelatine (10 per cent.) and ringworm organism and oil of wintergreen.

2. Gelatine and oil of wintergreen.
3. Gelatine and oil of wintergreen and liquefied gelatine from a ringworm culture.
4. Gelatine and ringworm organism.
5. Control tubes of uninoculated 10 per cent. gelatine.

The tubes were examined after having remained in an incubator at 30° C. for 18 hours. The only tubes that gave a positive result were those to which the "enzyme gelatine" had been added. The gelatine in these tubes was completely liquefied. The results corresponded with those obtained at blood heat, namely, complete liquefaction of the gelatine within 18 hours.

It was remarkable how small a quantity of the "enzyme gelatine" was necessary to produce liquefaction at 30° C. and at blood heat. Three droplets of "enzyme gelatine" caused a complete liquefaction of 10 per cent. gelatine at these temperatures.

The action of the "enzyme gelatine" was also tested on 15 per cent. gelatine. At blood heat, even this concentrated solution of gelatine was entirely liquefied.

If these results depend upon the presence of an enzyme in the gelatine, a temperature above 80° C. will interfere with or destroy its action. To test this, two tubes of gelatine, which had been completely liquefied by the trichophyton were used. One tube was exposed to a temperature of 100° C. for 2 minutes. A few drops of gelatine from the heated and unheated tubes were then added to respective tubes of fresh gelatine. The experiments with the heated gelatine gave entirely negative results. It produced no liquefaction of gelatine at 30° C. and at blood heat, though the tubes were left in the incubator for several days. On the other hand, the control tubes which had been inoculated with the unheated gelatine were liquefied.

The enzyme upon which the liquefying action on gelatine depends is thus rendered inactive when exposed to a temperature of 100° C. for 2 minutes.

A number of experiments were made with a simple 5 per cent. solution of gelatine in water, without any addition of peptone or beef-broth. The results were the same as in the previous experiments. The addition of a few drops of the "enzyme gelatine" produced a rapid, complete, and permanent liquefaction. This simple gelatine solution furnishes a very convenient means of rapidly testing the action of peptonising ferments generally.

Experiments were next made to determine whether the liquefying action of gelatine cultures of the trichophyton could be transmitted from tube to tube—whether, in other words, the enzyme would still act when greatly diluted. A few drops of the "enzyme gelatine" were added to sterile 10 per cent. gelatine, and the tubes placed at blood heat. When liquefaction was complete, a few drops were transferred to a second tube of gelatine. This second tube of gelatine was also liquefied at blood

heat. Some of the liquefied gelatine was then added to a third tube of gelatine, and so on. It was found that liquefaction still took place in the fourth tube of the series. This gave a fresh proof of the powerful nature of the peptonising enzyme secreted by the trichophyton, and of its capacity to act even when greatly diluted. The enzyme is also of a very stable nature. A few drops of gelatine from a ringworm culture 3 months old still exerted the liquefying action on gelatine.

These experiments seem to establish the following points. The *T. tonsurans* secretes a powerful peptonising enzyme, by means of which it is able to liquefy gelatine. The action of this enzyme can be demonstrated apart from the living cells that produce it. The enzyme rapidly liquefies 5, 10, and 15 per cent. solutions of gelatine. It can be greatly diluted without enfeebling its liquefying power. The enzyme is destroyed by boiling, but it is otherwise stable in its character, as it retains its activity for at least 3 months.

In the above experiments the action of the enzyme had been tested in neutral or alkaline media. Its action in acid media was next tested.

Nutrient gelatine was rendered acid by the addition of a 0.2 per cent. solution of hydrochloric acid. The trichophyton grew in this acid gelatine at 30° C., though its growth was slower than in neutral or alkaline gelatine. When liquefaction of the acid gelatine took place, a few drops were added to fresh gelatine. The tubes were kept at blood heat. After 2 days, the gelatine was partially liquefied. It was only on the third or fourth day that complete liquefaction took place.

The liquefied gelatine from an alkaline culture was added to the acidified gelatine. It was found that at blood heat complete liquefaction of this acid gelatine took place in 48 hours.

It will be seen that the peptonising enzyme is not only produced in an acid medium, but that it can also act in an acid medium. Its action, however, is slower and not so energetic as is the case when the medium has an alkaline reaction. It is undoubtedly sensitive to the presence of acids, and acts best when the medium is alkaline.

The *T. tonsurans* was next grown on fluid media, and the action of these cultures on gelatine was tested. Inoculations were made in a simple acid-broth infusion, and in a simple alkaline-broth infusion. In both cases a growth of the trichophyton was obtained. The broth was then added to a 5 per cent. solution of gelatine in water. The *alkaline* culture broth completely liquefied the 5 per cent. gelatine in 7 days. The *acid* culture broth produced no complete liquefaction of the 5 per cent. gelatine after the same lapse of time. The enzyme was therefore also produced in fluid broth cultures, and proved to be more active in the alkaline than in the acid broth. In this respect the results corresponded to those obtained with the acid and the alkaline gelatine. On the other hand, the enzyme produced in the alkaline gelatine was more energetic than that produced in the alkaline broth. The best

results were always obtained with alkaline gelatine cultures of the organisms.

Some experiments were made with a view of answering the following question:—Does the liquefied gelatine exercise any inimical action on other organisms in virtue of the enzyme which it contains? The experiments were carried out in the following manner. Gelatine which had been liquefied by the ringworm organism was boiled to destroy the ferment. A second series of tubes of the liquefied gelatine were not subjected to this heat. They therefore contained the active ferment. Both sets of tubes were then inoculated either with the *Staphylococcus pyogenes aureus* or the *Bacillus pyocyaneus*. Control cultures of these organisms were also made in ordinary nutrient gelatine. All the tubes were placed at blood heat. The results were negative as regards an inimical action of the enzyme on these organisms. The *Staphylococcus pyogenes aureus* and *B. pyocyaneus* grew well, not only in the control gelatine, but also in the heated and unheated tubes of "enzyme gelatine." One could not therefore demonstrate any inimical action on these bacteria, through the presence of the active ferment in the cultures.

Experiments were made to determine whether the *T. tonsurans* produces a *diastatic ferment*. The organism was grown in watery solutions of starch of various concentrations. A growth was obtained in the starch water. It was then tested for the presence of sugar. No unmistakable evidence of a conversion of the starch into sugar could be obtained. The experiments led to the conclusion that the trichophyton does not produce a diastatic ferment.

On the other hand, the trichophyton grew well in a 5 per cent. solution of grape sugar, and also in a 5 per cent. solution of milk sugar. It also grew well in beef broth containing 2 per cent. of milk sugar or grape sugar. These sugars seemed rather to favour its growth than otherwise.

The trichophyton developed also in a 2 per cent. solution of cane sugar in 1 per cent. peptone water. The growth, however, was due mainly to the presence of the peptone, and not to the cane sugar. In simple watery solutions of cane sugar, with the addition of mineral salts, a slight growth was obtained, but it was feeble compared with the growth in the solutions of grape sugar and milk sugar.

The cane-sugar solutions, after inoculation with ringworm, reduced Fehling's solution slightly. This at first pointed to the presence of a weak inverting ferment in these cultures.

In an abstract of these results, published in the *British Medical Journal* (Sept. 22, 1894), it was stated that evidence was obtained of the production of a weak inverting ferment by the trichophyton. The experiments have since then been repeated several times. It was found that the cane sugar used was not chemically pure. It was therefore recrystallised, and the experiments were then repeated.

In these pure solutions of cane sugar, the trichophyton again gave a very feeble growth. The Fehling's solution was not reduced. Further, an examination of the solutions with the polarimeter gave no evidence of the presence of invert sugar. The trichophyton cannot, therefore, be said to produce an inverting ferment.

Finally, experiments were made as to the production of a *milk-curdling ferment*. The results were negative. The ringworm did not grow at all in milk, nor did cultures containing the active enzyme produce any curdling of pure milk.

The experiments have thus demonstrated solely the production of a very active peptonising ferment by the *T. tonsurans*. The question naturally occurred whether this enzyme does not aid the organisms when it attacks the tissue, by perhaps overcoming their resistance to the penetration of the hyphæ.

The action of the peptonising enzyme was first of all tested on fibrin. The "enzyme gelatine," to which a little chloroform had been added, was placed in test-tubes containing flakes of fibrin. The tubes were kept at 20°, 30°, and 37° C. The fluid in the tubes remained clear, and no solution or disintegration of the fibrin took place. The enzyme did not act at all upon the fibrin.

It might be that the enzyme would, however, act on keratin or bodies containing it. Hairs from the human scalp were subjected to the action of the peptonising enzyme. These experiments did not lead to any definite result. The action of the trichophyton and its ferment were finally tested on pure keratin. The keratin used was obtained from quills, and answered the following tests:—It was insoluble in water, dilute acids, dilute alkalis, alcohol, and ether. The first point was to ascertain whether the trichophyton would grow on a keratin soil. For this purpose a thick layer of keratin was placed on the surface of solid blood serum. The tubes were then sterilised. The keratin surface was then inoculated with the trichophyton, and the tubes incubated at 30° C. A growth was obtained under these conditions. The *T. tonsurans*, therefore, was capable of growing on a soil consisting entirely of keratin, and was apparently able to derive its nutriment from the keratin. One could not, however, observe that any solution of the keratin took place. The ringworm certainly grew on the keratin, but did not exhibit any dissolving action that might be attributed to the action of an enzyme.

Gelatine containing the active enzyme was added to the pure keratin, and here again no distinct evidence of solution of the keratin was obtained.

The growth of the trichophyton might, however, possibly affect the solubility of the keratin in some way. The keratin might, for example, be rendered more easily soluble than it is under ordinary conditions. Tubes of nutrient gelatine, containing keratin, were inoculated with the trichophyton, and incubated at 30° and 37° C. The organism grew equally well in the keratin-gelatine.

Control tubes of gelatine and keratin alone were also kept under the same conditions. After a good growth had appeared in the inoculated tubes the keratin was removed from the tubes, and its solubility, in a strong solution of caustic potash, was tested. The keratin from the ringworm tubes dissolved immediately on the alkaline solution being heated. The keratin from the control tubes required a prolonged boiling to dissolve it. The solubility of the keratin seemed, therefore, to have been affected by the growth of the trichophyton in the gelatine. This was as far as the experiments with keratin could be brought. The writer does not wish to lay too much stress on these experiments. They certainly suggest that the solubility of the keratin is affected, and the results seem worthy of being tested by other workers in the same field. At anyrate, it was interesting to note the fact that the trichophyton was capable of growing on a purely keratin soil.

It may be mentioned here that the trichophyton used in the above experiments was generally grown on the beer-wort agar, recommended by Dr Sabouraud. This medium furnishes an excellent soil for the *T. tonsurans*. It is prepared as follows:—Add 1–5 grms. of agar-agar to 100 grms. of beer-wort; then boil and filter hot. Place the filtrate in test-tubes or Erlenmeyer flasks, and sterilise in the usual way. The beer-wort agar solidifies on cooling.

VARIABILITY OF THE "COMMA BACILLUS" AND THE BACTERIOLOGICAL DIAGNOSIS OF CHOLERA.

*BASED ON THE STUDY OF SOME CASES WHICH OCCURRED IN
MANCHESTER AT THE END OF THE SUMMER 1893.¹*

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(PLATES XIII. AND XIV.)

I. PRELIMINARY REMARKS.

WHEN we attempt to compare the records of recent epidemics of Cholera Asiatica with those of epidemics which occurred before 1884, we cannot help wondering whether or not the records are comparable.

Since Koch's important discovery in 1884, the basis of diagnosis has become more and more bacteriological, and for the last few years it may be said that, in Europe at least, more importance has been attached to the discovery of the comma bacillus than to those epidemiological and clinical features on which, previously, the diagnosis of Asiatic cholera had rested.

At the same time, it has been discovered that Asiatic cholera has apparently become endemic in several countries, and that small insignificant outbreaks have occurred in places widely distant from each other.

In some instances, indeed, it is very difficult to find out the exact mode of infection of communities.

One may, therefore, logically ask, Has the disease altered in its type, or have we by our new diagnostic methods discovered a prevalence of cholera hitherto unsuspected?

Another tendency worthy of remark has become manifest of late. Whilst, on the one hand, the proportion of cases of cholera, in which the comma bacillus has been found, has become greater and greater, on the other, the differential characters by which the comma bacillus can

¹ This paper is a fuller *exposé* of the views advanced by one of us at a meeting of the Manchester Medical Society, December 1893 (S. Delépine, *Brit. Med. Journ.*, London, January 20, 1894).

be distinguished from allied vibrios have become fewer and less distinct, notwithstanding the introduction of new methods of diagnosis, some of them, like the cholera-red reaction, of paramount importance.

These considerations naturally lead one to ask, Which is the variable element—the disease or the diagnosis? All agree that the disease is variable, but this is not enough to prove that diagnosis has nothing to do with the change to which we have alluded. Whatever importance we may attach to Koch's discovery, it is surely false logic to assume what we wish to prove; yet in some papers statistics of cholera, of which the diagnosis is based chiefly on the presence of cholera spirilla, have been used to prove that the cholera spirillum is almost invariably present in the intestines of cholera patients. Is this truly scientific? The danger of such a procedure is well shown by the changes which have taken place in the methods of bacteriological diagnosis as applied to cholera during the last ten years.

At first the diagnosis was based upon—

1. The shape of young colonies in plate cultivations.
2. The rate of liquefaction of gelatine in plate and tube cultivations.
3. The shape of the bacillus, vibrio, or spirillum (according to the view one may take of its morphology).
4. Its behaviour in drop cultivations.
5. The absence of changes produced in milk.
6. In 1887 another test was given, the production of cholera-red.
7. Then a seventh test, viz. the virulence as tested in guinea-pigs was added.

Two additional tests have also been proposed by some, viz:—

8. The production of immunity against infection by a genuine cholera bacillus.
9. The duration of this immunity, produced either by direct inoculation or by inoculation of the serum of immunised animals.¹

None of these differential characters has wholly stood the tests of time and critical examination, with perhaps the exception of the characters presented by the young colonies on gelatine plates from the seven-teenth to twentieth hour.

Taken in combination the following characters, viz.—

- (a) The human origin,
 - (b) The shape and appearance of young colonies,
 - (c) The rapid production of cholera-red in aqueous saline solution of peptone at 37° C., and to a certain extent,
 - (d) The virulence of the vibrio,
- may be said to have gradually acquired more and more importance.

When, however, we come to the diagnosis of the cholera spirillum outside the human body, it may be said that our task is becoming less

¹ We purposely avoid mentioning certain cultural characters, such as the production of a film in bouillon cultures, and that of pigment in potato cultivations, on account of the secondary importance which they have, at anyrate, in our mind.

and less easy, although the methods have vastly improved of late. That we do not exaggerate the difficulties is readily seen on reference to the literature of this subject.

II. SHORT OUTLINE OF SOME OF THE MOST IMPORTANT PAPERS BEARING DIRECTLY OR INDIRECTLY ON THE SUBJECT.

1. *In various Epidemics of Cholera, Choleraic Cases have been described in which Cholera Spirilla have not been found.*

D. Cunningham¹ mentions such cases.

Gruber² mentions 9 fatal cases.

Lesage and Macaigne³ give 33 fatal cases.

Netter⁴ gives 10 cases, 4 of them fatal.

Girode⁵ gives 11 cases, 5 of them fatal.

Girandau and Rénon.⁶—2 fatal cases.

Kirchner.⁷—16 cases, with 3 deaths.

Beck.⁸—1 fatal case.

Fischer.⁹—1 fatal case.

Guttmann.¹⁰—1 fatal case.

Furbringer,¹¹ at the Wiesbaden Congress, said he had met with such cases in 1887 and 1892, and noted their frequency before and during cholera epidemics.

Rumpf,¹² at the same Congress, mentioned several cases occurring in Hamburg, in January 1893.

Rumpel¹³ had 3 cases of this kind which recovered in the second epidemic, 1892–93, at Hamburg.

Carp.¹⁴—3 cases, examined bacteriologically, with 2 deaths.

Renvers¹⁵ grouped cases under his care in 1893, and in which the cholera spirillum had not been found, into three classes—(a) In which *enteritis acutissima*, without fever, was present with all the symptoms of cholera, and in these cases other bacilli and streptococci were found. These were cases of poisoning by bad meat, etc. (b) In which there was fever with symptoms of cholera. In these cases no special forms of bacteria were predominant. (c) In which there were gastro-intestinal troubles from dietetic errors. These cases soon recovered.

¹ "Scientific Memoirs of Medical Officers of the Army of India," Calcutta, 1891, p. 36.

² *Wien. med. Wchnschr.*, 1887, ss. 185, 221.

³ *Ann. de l'Inst. Pasteur*, Paris, 1893, p. 18.

⁴ *Progrès méd.*, Paris, 1892, tome ii. p. 665.

⁵ "Mémoires de la Soc. de Biologie," 1892, s. 295.

⁶ *Gaz. hebdom. de méd.*, Paris, 1893, p. 558.

⁷ *Berl. klin. Wchnschr.*, 1892, s. 1073.

⁸ *Deutsche med. Wchnschr.*, Leipzig, 1892, s. 902.

⁹ *Ibid.* 1892, s. 902.

¹⁰ *Ibid.* 1892, s. 1020.

¹¹ *Ibid.* 1893, s. 381; *Semaine méd.*, Paris, 1894, p. 174.

¹² *Ibid.* 1893, p. 173.

¹³ *Deutsche med. Wchnschr.*, Leipzig, 1893, s. 160.

¹⁴ *Ibid.* 1893, s. 34.

¹⁵ *Ibid.* 1894, s. 52.

Balster¹ had a case in which no comma bacilli were present on the first day (examination, in Koch's laboratory, by Koch personally), in the slightly bloody stools when the algid stage was imminent.

In connection with these it may be well to keep in mind Wiltshur's observations.² This author found in 70 cases of cholera, which he examined bacteriologically, short bacilli staining well at both ends;—not so well or not at all in the middle. In all these cases but three he also found comma-shaped bacilli. By cultivating those short bacilli at 37°, this author says that the small bacilli, after 10 or 12 generations, assumed the characters of typical comma bacilli. At first these short bacilli liquefied gelatine rapidly, but after they had taken the comma shape they liquefied gelatine more slowly. Although we mention this paper here, we cannot help feeling that the evidence brought forward is not convincing.

2. *When Cholera is epidemic, Cases without any symptoms of Cholera may occur in which Cholera Bacilli are found in the stools.*

Rumpel³ in the second epidemic at Hamburg, found comma bacilli in the normal stools of healthy persons.

Renvers⁴ had at Hamburg, in the autumn of 1893, cases in which comma bacilli were found in persons who did not present the clinical signs of cholera.

Metchnikoff⁵ found the comma bacillus, in the absence of epidemic cholera, in the stools of a healthy person who drank mineral water only.

Though not, strictly speaking, belonging to this category, those cases in which infection in man has been attempted may be mentioned. In some of these no pathogenic action has been produced, and the comma bacilli have been cultivated from the normal stools.⁶

3. *The Drinking Water has been found to contain Bacilli, resembling the Cholera Bacilli in several places where Epidemic Cases of Cholera had occurred.*

Koch⁷ found them in great numbers in an Indian tank when cholera prevailed in the neighbouring huts.

Guarch,⁸ at Monte Video, in January 1887, found comma bacilli in a water butt, from which persons who suffered from cholera had drawn

¹ *Deutsche med. Wchnschr.*, Leipzig, 1893, s. 913.

² *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, bd. xv. s. 158.

³ *Ibid.* Jena, bd. xv., 1893, s. 162.

⁴ *Ibid.* 1894, s. 52.; *Semaine méd.*, Paris, 1893, p. 559.

⁵ Quoted by Sanarelli, *Ann. de l'Inst. Pasteur*, Paris, 1893, p. 693.

⁶ Hasterlik, "Cholera-Experimente am Menschen" in Stricker's "Studien zur Cholerafrage," Wien, 1893; Metchnikoff, in the *Ann. de l'Inst. Pasteur*, Paris, 1893, p. 562 etc.

⁷ *Deutsche med. Wchnschr.*, Leipzig, 1884, No. 45.

⁸ *Baumgarten's Jahresh.*, 1888, s. 284.

their supply of water. The well from which this vessel was filled did not yield any vibrios on examination.

Müller¹ reports a number of cases at Havelburg, a coaling-station on the waterway between Hamburg and Berlin. All affected had used the river water. This was afterwards found to contain comma bacilli. Cholera was prevalent at the time at Hamburg.

Biernacki² records an outbreak in a house at Lublin among persons who used water from a well contaminated from a cesspool. The water was rich in comma bacilli.

C. Fränkel³ found a pathogenic comma bacillus growing like Koch's, *but not giving the cholera-red reaction*, in the water of the Zollhafen at Duisberg. A fatal case of cholera, proved bacteriologically, had occurred on board a ship which lay in this harbour, and another case arose in Duisberg.

Koch⁴ found cholera bacilli in a well, in Altona, which was liable to contamination from slop-water, etc. Cholera prevailed among those who used this water, and ceased when the well was closed. In the Nietleben outbreak cholera bacilli were found in the water of the Saale below the effluent outfall of the sewage works, in the mud of the filter at the waterworks, in the water-supply of the asylum, in the sewage of the asylum, in the filter-beds of the sewage works, and in the sewage effluent, thus completing the circuit. At a number of places on the Saale, below Nietleben, some who drank the water of the Saale had cholera. At each of these places cholera bacilli were found in the river. During the winter outbreak at Altona,⁵ these bacilli were found in the Elbe, below Hamburg, in the Altona water before filtration, and in both reservoirs of filtered water at the Altona waterworks.

Loeffler⁶ found comma bacilli in the water pail used at a house in Demmin, where a fatal case of cholera had occurred. The supply usually came from a well, which when examined a few days later yielded no comma bacilli. The persons in the house had also used water from a ditch supplied from the foul river Peene, the water of which contained vibrios like Finkler's; more slowly liquefying vibrios growing like Koch's were also found.

Barwise⁷ records an outbreak at Ashbourne, in September 1893, among persons whose water supply was derived from a well contaminated with faecal matter from an adjoining closet. The water of this well, examined by Klein, was found to contain cholera bacilli.

Fischer⁸ records the examination of 7 cases of cholera in men engaged on a dredging-machine on the Eider Canal. Vibrios of the

¹ *Berl. klin. Wchnschr.*, 1892, s. 1145.

² *Deutsche med. Wchnschr.*, Leipzig, 1892, s. 957.

³ *Ztschr. f. Hyg.*, Leipzig, bd. xv. s. 89, etc.

⁴ *Op. cit.* bd. xiv. p. 393, etc.

⁵ *Deutsche med. Wchnschr.*, Leipzig, 1893, s. 263.

⁶ *Brit. Med. Journ.*, 1893, vol. ii. p. 909.

⁷ *Deutsche Med. Wchnschr.*, Leipzig, 1893, s. 542, etc.

⁸ *Ibid.* 1892, s. 925.

classical type were found in the sludge dredged at four different places on the canal. Similar vibrios have been found by Spronck¹ in river, canal, and gutter water in Holland, and by Mendoza² in various river waters in Spain. Vibrios, more or less like cholera vibrios, have been found in water near Magdeburg, in Halle, Wittenberg, Berlin, Ruhrort, Stettin, and Amsterdam after cases of cholera had arisen in these places.³

Lubarsch⁴ found cholera bacilli in the bilge water of an Elbe tug-boat on which a case of cholera had occurred.

Pasquale⁵ found comma bacilli in a well at Massowah.

4. Cases in which Comma Bacilli like Koch's have been found in the Drinking Water or Sewage of Communities who were not suffering from Cholera at the time.

Klein,⁶ in an Indian tank, at a time when the persons using the water were free from cholera.

Sanarelli⁷ found spirilla which gave the cholera-red reaction and were pathogenic to guinea-pigs in the Seine, the sewage effluent at Gennevilliers, and in the drinking-water at Versailles, at a time when there was no cholera in Paris or Versailles.

Spirilla like Koch's were found in the Elbe at Hamburg by Dunbar⁸ six weeks before cholera appeared in Hamburg, and then a case was imported from Rotterdam.⁹ In 1893, according to Dunbar, such spirilla were found in the Elbe near Dresden, in the Unstrut (Naumburg) and in the Pegnitz (Nurnberg), although cholera was not found at these places in that year.¹⁰

Neisser¹¹ isolated from the Berlin tap-water a comma bacillus (*Vibrio berolinensis*) which gave the cholera-red reaction and was pathogenic to guinea-pigs. Colonies, 24-48 hours' old, are more translucent than Koch's, are more finely granular, with a smooth regular circular margin as a rule. Older colonies have a brown colour, are covered with irregular elevations finely granular, often disposed radially. Gelatine is liquefied slowly. At that time the only cases of cholera in Berlin had been imported or had occurred in persons who had drunk water directly from the Spree or the Havel.¹²

¹ *Centralbl. f. d. med. Wissensch.*, Berlin, 1894, s. 102.

² *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, bd. xiv. s. 693.

³ Dunbar, *Deutsche med. Wchnschr.*, Leipzig, June 21, 1894; *Literatur Beilage*, s. 10, from "Arbeiten a. d. k. Gesundheitsamte."

⁴ *Deutsche med. Wchnschr.*, Leipzig, 1892, s. 978.

⁵ *Riforma med.*, Roma, 1892, vol. i. p. 310.

⁶ *Practitioner*, vol. xxxviii. p. 279.

⁷ *Loc. cit.*

⁸ *Deutsche med. Wchnschr.*, Leipzig, 1893, s. 779.

⁹ *Eodem loco*, p. 1301.

¹⁰ Dunbar, "Arbeiten a. d. k. Gesundheitsamte," *op. cit.*

¹¹ *Arch. f. Hyg.*, München, 1893, Rubner, *Hygienische Rundschau*, 1893, No. 16.

¹² Renvers, *Semaine méd.*, Paris, 1893, p. 559.

Heider¹ isolated from the Danube a comma bacillus (*V. danubicus*), very similar to the *V. berolinensis* in its properties. By cultivation in the laboratory for some time its liquefactive power is now equal to that of Finkler's vibrio.²

Of these vibrios Dunbar³ considers the *V. berolinensis* and the *V. danubicus* alone to be like the cholera vibrio, from which in their culture and pathogenic action they are indistinguishable. Russell's, Gunther's, Loeffler's, Bonhoff's, Sanarelli's, and Fokker's vibrios are, according to him, not like those of cholera.⁴

5. Variations of the Comma Bacillus.

(A) In various epidemics or places.

Zäselein⁵—In simultaneous epidemics in Genoa, Palermo, and Naples, the spirilla isolated at each place behaved in a distinctive way in plate cultures and tube cultures.

Netter⁶ found the comma bacillus of the Paris (1892) epidemic was shorter and thicker than Koch's, and coagulated milk.

Finkelnburg⁷ found that the spirilla of Hamburg and Paris (1892) differed from Koch's in being thicker and more swollen in the middle, having also a greater tendency to assume spirillar forms; and in showing a greater resistance to cold and to acids, a more intense acid fermentation of lactose, and a greater power of destroying red blood cells. They differed but slightly from each other in rate of growth and liquefaction of gelatine.

Sclavo⁸ examined spirilla from outbreaks in Cochin-China, Ghinda, and Massowah, in comparison with three specimens from Paris and one from India. The African form was less curved than the rest, and did not constantly give the cholera-red reaction, and was the only form giving a pellicle on sugar bouillon. It is very virulent to guinea-pigs. The Indian bacillus gave the greatest acid fermentation in sugar bouillon, next came the Paris form and the Cochin-China form; that from Africa gave less by half. The Indian form grew on potato only at 37° C.; the rest at ordinary temperatures.

Nicolle and Morax⁹ classified the flagellated cholera vibrios according to the number and position of their flagella into two types—(1) having one flagellum at one end or at each end, as in specimens from Shanghai,

¹ *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, bd. xiv. No. 2.

² Gruber, *Lancet*, 1894, vol. ii. p. 6.

³ *Op. cit.*

⁴ One of us (J. Richmond) in attempting to isolate spirilla resembling Koch's spirillum from the Manchester sewage in February last, found no organism that could be mistaken for Koch's bacillus.

⁵ *Deutsche Med.-Ztg.*, Berlin, 1888, Nos. 64, 65.

⁶ *Progrès méd.*, Paris, 1892, tome vi. p. 65.

⁷ *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1893, bd. xiii. s. 113.

⁸ *Jahresb. ü. d. Leistung . . . d. ges. Med.*, Berlin, 1892, bd. ii. s. 31.

⁹ *Ann. de l'Inst. Pasteur*, Paris, 1893, p. 555.

Hamburg, Courbevoie, and Angers; (2) having four flagella, two at each end, or one at one and three at the other end, as in bacilli from Calcutta, Massowah, and Paris (1884). Another specimen from India was not motile and had no flagella. These types were preserved after passage through animals.

Sirena¹ found many differences in specimens obtained in the same year from Palermo, Rome, Naples, and Calcutta, in form, length, thickness, and motility in drop cultures. Gelatine was liquefied quickest by that from Palermo, next in order came those from Naples, Rome, and Calcutta. The potato cultivations and the rate of coagulation of very alkaline milk were different. Those from Rome and Naples gave the cholera-red reaction quickest; next in order was that from Palermo, whilst the Indian gave it very slowly or not at all. Those from Palermo, Rome, and Naples killed guinea-pigs in 12 hours, the Indian in 15–20 hours.

Metchnikoff² recognises two main types of cholera vibrios.

(1) Short curved vibrios, like that of Koch and that found at Angers.

(2) Long thin filaments, sometimes almost straight and sometimes in spirals of several turns. (Paris 1884, Massowah, and Courbevoie.)

Type 1 is changed into Type 2 by passage through immunised guinea-pigs, or by growth on agar containing blood serum from these animals, and Type 2 changes into Type 1 temporarily in old cultivations. He finds that undoubted cholera vibrios, when grown on the usual 10 per cent. gelatine, show sometimes sharply outlined, finely granular colonies at an early stage, in the place of the usual coarsely granular colonies with irregular margins.

(B) *In various cases.*

Zäselein,³ in 1886, in Genoa, in some cases found bacilli of the Finkler's type.

Gruber⁴ had similar cases in 1886.

Cunningham⁵ isolated ten "*kinds*" of comma bacilli from cases of cholera at Calcutta. They differed in shape and mode of growth in different media. *One form* did not give the cholera-red reaction; in the original colonies it was markedly curved and large; long spirilla were present; the gelatine was not liquefied. The other forms gave the cholera-red reaction more or less readily, liquefied gelatine at various rates, and were of various shapes. Subsequently⁶ he describes four other "*species*" of cholera spirilla, and notes the change his "*species*" have undergone in artificial media since their isolation. These

¹ *Centralbl. f. Innere Med.*, 1894, s. 579.

² *Ann. de l'Inst. Pasteur*, Paris, 1894, p. 287.

³ *Deutsche Med.-Ztg.*, Berlin, 1887, No. 23.

⁴ *Wien. med. Wchnschr.*, 1887, ss. 188, 221.

⁵ "Scientific Memoirs of the Medical Officers of the Army of India," pt. vi. p. 1, Calcutta, 1891.

⁶ *Op. cit.* pt. viii. p. 1, 1894.

changes do not tend to reversion to a common form but to diversity of type. The form which did not give cholera-red still fails to give the reaction. The reaction is still given by the rest.

Napsal¹ found vibrios of the Finkler type in the contents of the ileum, in 3 cases of suspected cholera.

Friedreich² examined comma bacilli from many sources, including Cunningham's forms, and confirmed some of Hueppe's observations, to be mentioned later on. He found that six out of seven of Cunningham's forms killed guinea-pigs by Koch's method.

Fischer³ described some cholera bacilli which gave colonies like Finkler's.

Canon, Pielicke, and Lazarus⁴ found comma bacilli in the intestinal contents of some cases of suspected cholera, which, in gelatine cultures, differed from those of Koch.

Hueppe⁵ says cholera bacilli do not always grow as Koch described them.

Lesage and Macaigne⁶ found a great variability in the comma bacilli they isolated from their cases of cholera, so that, for their classification, six divisions would be necessary.

Zörkendörper⁷ found, in cholera stools, a vibrio like Koch's pathogenic to guinea-pigs, but liquefying gelatine more quickly and not giving the cholera-red reaction.⁸

(C) *In the same case.*

Here the evidence is not abundant, and it is even doubtful whether the authors to whom we allude had in their mind the question of variability, to which one of us attaches special importance.

¹ *Gaz. hebdomadaire de médecine*, Paris, 1893, p. 227.

² "Arbeiten a. d. k. Gesundheitsamte," 1893, bd. viii. s. 87.

³ *Deutsche med. Wochenschr.*, Leipzig, 1893, s. 541, etc.

⁴ *Loc. cit.*

⁵ *Berl. klin. Wochenschr.*, 1893, s. 81, etc.

⁶ *Op. cit.*

⁷ *Prag. med. Wochenschr.*, 1893, No. 44.

⁸ Vogler,¹ in the stools of a patient, living in an infected place, who suffered from diarrhoea and delirium, found a motile vibrio which gave finely granular colonies, having dark homogeneous centres in 48 hours; other colonies had irregular margins with dark central and clear peripheral parts; a third set of colonies had granular contents within an area of liquefaction, which was bounded by a zone of radiating fibrils. These vibrios did not give the cholera-red reaction, and were not pathogenic to guinea-pigs. Fischer² found in the stools of a non-fatal case with choleraic symptoms, which had come on 7 days after nursing a case of severe "diarrhoea with cramps," a vibrio thicker and more curved than Koch's; it has a flagellum at one end. Colonies in 24 hours resembled Finkler's, but were darker and liquefied gelatine more rapidly; cholera-red reaction very slight. Milk was curdled in 1-2 days at 37°. It was very virulent to guinea-pigs. Subcutaneous injection gave rise to abscesses. Hence the name *Vibrio helkogenes*. Such cases are mentioned here only as having a distant connection with the subject, since the microbes referred to but little resemble Koch's bacillus.

¹ *Deutsche med. Wochenschr.*, Leipzig, 1893, s. 836.

² *Ibid.* 1893, s. 541, etc.

Lustig¹ in 2 cases found, on the same plate, colonies slowly liquefying like those of Koch, with quickly liquefying colonies like those of Finkler, but he did not say that the latter were colonies of cholera bacilli.

Cunningham² obtained three of his so-called "species" from 1 case.

Fischer³ often found colonies more rapidly liquefying than the typical form present on the same plate with typical forms.

The remarkable differences in the characters of true cholera spirilla, isolated by one of us in some suspected cases of cholera, impressed him so much that he determined to find whether that variability was capable of vitiating the bacteriological diagnosis of the disease.⁴ This, of course, might occur if the bacilli were capable of assuming the appearances of organisms which are commonly found elsewhere than in cholera cases, and if other organisms not connected with cholera could be mistaken for the cholera bacillus.

The study of the literature, of which a short abstract only has been given here, has impressed us with the fact that we are not as yet in a position to be dogmatic upon this question.

As we have already said, whilst evidence is being accumulated proving the all but constant presence in cholera of comma bacilli, identical with or closely allied to that originally described by Koch, it becomes equally evident that the rigid monomorphism attributed to the comma bacillus by Koch must be abandoned, and that we must admit that this organism is variable in its characters.

The only scientific solution of the question depends, in the first place, on an accumulation of data which may be compared with one another. To obtain such data, observers, under whose notice small outbreaks of choleraic disease occur, especially in what has been called the marginal zone of epidemic areas, should record carefully the results of their bacteriological investigations of the cases in such a way as to make them comparable with the results of other observers. Many have already done this, but others have given results which, however interesting, are not altogether as useful as they might be.

At the same time, it is right to mention that many of the results obtained before the cholera-red reaction was adopted as a test can hardly be said to be comparable with those obtained during the last two or three years; for at the earlier period the rate of liquefaction of gelatine and the shape of the bacillus were the chief diagnostic features.

The only observations which can be said to be comparable all through the last ten years are those based on the examination of the young colonies so well described by Koch ten years ago.

¹ *Centralbl. f. d. med. Wissensch.*, Berlin, 1887, s. 361.

² *Op. cit.*

³ *Op. cit.*

⁴ *Op. cit.*

III. MANCHESTER CASES OF CHOLERA, DURING THE MONTH OF SEPTEMBER 1893.

We are indebted to Dr. Tatham (then Medical Officer of Health for Manchester) for kindly allowing us to use some of the information which he was able to obtain at the time of the outbreak from Drs. Hackett, Orchard, and Sutherland, who had charge of the cases, and by personal visitation of the houses affected.

The history is briefly as follows:—

CASE A—1. Clinical features.—A married woman, *æt.* 44 years, was taken ill with purging of rice-water stools, cramps, and vomiting, on the 30th August 1893 (another account gives the date September 5th). She was removed to hospital on September 14th, and died on the 15th, after an illness lasting certainly more than 9 days. Dr. Hackett, who examined her at home, found all the symptoms usually prevalent in Asiatic cholera. Dr. Orchard, who saw her in the hospital 12 hours before she died, says “she was cold, unconscious, collapsed; her face presented a ‘pinched’ appearance, and she had dark rings round the eyes.”

2. Evidences of infection.—Drs. Hackett and Tatham found on inquiry that, 2 days before falling ill, the patient, with her husband and a friend, had partaken to an unwise extent of oysters. These oysters had come from Grimsby, a place at that time infected with cholera. Neither the husband nor his friend suffered, but the mother-in-law of the patient, who slept with her, fell ill soon after the patient (see Case B).

3. Conditions of life.—The patient and family (mother-in-law and husband) occupied two rooms in one of the poor, crowded districts of Manchester (West Gorton). She was nursed by her husband.

The house was reported to be well drained, and no grave insanitary condition was noticed.

The milk supply gave rise to no suspicion. The water supply was good.

No special precautions for isolation or disinfection were taken until the day before death, when Dr. Hackett first saw and notified the case (*i.e.* for at least 7 or 8 days).

4. Post-mortem appearances (24 hours after death).—*Jejunum*—Slightly congested; contents, pale, yellow, pappy; smell, offensive, *fæcal*. *Pleum*—Mucous and serous coats generally congested, but more especially so in patches; Peyer's patches indistinct, no hæmorrhages; contents, very thick, adhesive, mucous, partly yellow and partly bright green, containing a few whitish flakes.

Microscopically, the whitish flakes were found to consist of numerous columnar epithelium cells; large, thick, and long bacilli (resembling *B. subtilis*); short, plump, straight, or very slightly curved bacilli (*B. coli* type) and micrococci; all these organisms being abundant. In addition, some large torula-like organisms were found, and a few slender curved bacilli *having the characters of the comma bacillus, but nowhere arranged in a typical manner.*

The lungs were deeply congested, and contained hæmorrhagic blocks, especially under the pleura.

The liver was dark and congested.

The kidneys showed typical cloudy swellings of the cortex with small areas of congestion, especially under the capsule; the medulla was intensely congested.

5. Bacteriological examination (September 16, 1893):—

(1) *Gelatine plate cultivations.*—The following colonies were found to be the most abundant:—

Non-liquefying colonies—

(a) Colonies of the *B. coli* type.

Liquefying colonies—

(b) Colonies of long bacilli, liquefaction rapid.

(c) Colonies of small curved bacilli, some slender and some thick, liquefying gelatine at various rates (Colonies *a*, *b*, *c*).

In Colonies *a* the liquefaction was already advanced in 24 hours, so that, as they did not agree with Koch's description, they were not further studied. (From further observations, it is probable that these were cholera bacilli.) (See Plate XIII. Type I. Figs. 13, 15.)

In Colonies *b* the gelatine was slightly liquefied at the end of 24 hours; in other respects they resembled closely the colonies of the comma bacillus at the end of 48 or 72 hours. (See Plate XIII. Type II. Figs. 9, 10, 11, 12.)

In Colonies *c*, at the end of 20 and 24 hours, were found the typical appearances described by Koch—the sharp, irregular outline, the wavy surface, the slight yellow colour, the uniform, coarsely granulated, and at the same time clear appearance. Some of these were extremely small, and had not, even at the end of 48 hours, begun to liquefy the gelatine. They, however, retained their typical appearance as they grew larger, and began to liquefy the gelatine before the end of the third day. (See Plate XIII. Type IV. Figs. 1, 2, 3, 4.)

A few colonies of other organisms were obtained at a later period in the second and third dilutions, but were not studied.

(2) *Cultivations in peptone salt water.*—At first nothing approaching a pure cultivation of comma bacilli was obtained. At the end of 20 hours, comma bacilli could be detected in the upper strata, mixed with a number of bacilli having the characters of *B. coli communis*. A faint pink tinge was produced on adding two or three drops of pure sulphuric acid to about five c.c. of the culture, but no pure cholera-red reaction was produced. Gelatine plate cultivations of the superficial strata gave, at the end of 20 hours, typical colonies of cholera bacilli, especially in the second and third dilutions, with a great excess of colonies of *B. coli communis*. From these plates it was easy to get pure cultivations in peptone water, which gave in 6 hours a faint, and in 24 hours an intense, cholera-red reaction. From the same plates pure cultivations on gelatine and agar were made.

(3) *Agar cultivations* from the first peptone tube gave almost pure cultures of an organism of the *B. coli communis* type.

(4) *Pathogenic action.*—One-third part of a pure cultivation on agar injected into the peritoneum of a guinea-pig weighing a little over 300 grammes (4 days after the death of the patient from whom the vibrio was obtained) proved fatal in 20 hours.

(5) In drop cultivations in bouillon, typical commas, S forms, and spirilla, with two or three turns, were easily obtained, but long spirilla were rare.

(6) The growth on potatoes was not satisfactory, owing probably to the unusual degree of acidity of the specimens available; for, after steeping the slices of potato in alkaline bouillon, a moist pale brown growth was easily obtained.

CASE B—1. *Clinical features.*—The mother-in-law of Case A fell ill about the same time as A, the symptoms, however, being less severe. The diarrhoea at first was considerable, but in about 9 days from the onset of the illness the stools were only three daily, with very little pain or cramp. The patient was at that time warm and comfortable, and the aspect was not typical of cholera. She recovered very slowly, remaining weak for some time.

2. *Evidences of infection.*—She slept with Case A before her removal to hospital, but had not been exposed to the same source of infection as A (see Case A).

3. *Conditions of life* (see Case A).

4. *Examination of stools*.—Fæces examined a few hours after passage were thin, pulpy, pale greyish-yellow, with whitish curdy masses or flakes; smell very fœtid and fæculent. *Microscopically*, the whitish flakes contained very few distinct epithelial cells; a large amount of granular débris; long thin bacilli; short plump bacilli (*B. coli* type) very abundant; streptococci, curved bacilli resembling closely the comma bacillus, *but nowhere forming typical groups*.

5. *Bacteriological examination*.—Gave practically the same results as Case A:—(1) A very great number of colonies of *B. coli communis*; (2) a smaller number of colonies having all the characters of colonies of the *cholera bacillus*, from which typical cultivations in gelatine, peptone water, agar, and bouillon could be obtained. Other organisms were observed, but were not studied so carefully as to justify their mention here.

CASE C—1. *Clinical features*.—The patient was a married woman, æt. 53. She was taken ill on the 14th or 15th of September, but remained at her work till the 15th, when in the morning she was seized with severe vomiting, diarrhœa, and cramps. The stools were flaky and colourless. Dr. Sutherland found her collapsed, bluish-coloured, and cold. She could not speak, but muttered indistinctly. She died the same evening.

2. *Evidences of infection*.—The husband of the patient had a choleraic attack on the morning of the 11th on his return from Grimsby (an infected town). Three days after, C fell ill. No other case arose in the house or in the neighbourhood.

3. *Conditions of life*.—C lived with her husband and a daughter-in-law in two rooms of a small four-roomed house in a crowded district (Ancoats), where the general sanitary conditions are not satisfactory, and the rate of mortality high. The daughter-in-law nursed the patient, and did the house work. She escaped infection. The house was clean, well drained, and no grave insanitary conditions were found on the 16th September 1893. The water supply was good. The various milk supplies excited no suspicion. The case was notified on the 16th of September, no precautions to prevent spread being taken until that date. Attached to the house was a pail closet, used only by the family and provided with a good tub, cleared once a week.

4. *Post-mortem examination*.—*Ileum* showed hardly any change except slight congestions of the serous coat. The contents were very thin and watery, pale brownish-grey, almost colourless. The more solid parts consisted of whitish threads and masses of mucus resembling boiled sago. Smell only slightly fæcal. *Microscopically*, the contents were much poorer in cells and bacteria than in Case A. The most important micro-organisms were some of the *B. coli communis* type, small cocci, some large bacilli, and some bacilli like those of cholera but nowhere in typical groups.

5. *Bacteriological examination*.—As in the other cases an organism of the *B. coli communis* type predominated, but typical cultivations of the comma bacilli were easily obtained from the first plate. Only on the third day was a good cholera-red reaction obtained (the failures on the second day were probably due to contamination). The first peptone cultivations from the stools were impure up to the surface layers, and an unequivocal cholera-red reaction was obtained only by separation, on gelatine plates, of the comma bacilli from the other organisms. The same variations in the rate of liquefaction of gelatine by various colonies were noticed in this as in the other cases. One type (Type III.) only was kept for further investigation. It was characterised by moderately slow liquefaction. One-sixth to one-fifth part of an agar culture, 20 hours old, 4 days after the death of the patient, killed a guinea-pig, weighing about 300 grammes in 18 to 20 hours. From the peritoneal exudation of the guinea-pig pure cultures were obtained, which gave an intense cholera-red reaction.

CASE D—1. *Clinical features*.—A workman, æt. 60, husband of C; fell ill on 11th September with diarrhoea, vomiting, and cramps, and became very weak. On the 20th he was very prostrate, diarrhoea still well marked but not so severe; he felt a little easier. After a slow convalescence he recovered.

2. *Evidences of infection*.—He left Manchester on the 2nd September, reached Grimsby on the 5th, and lodged near the docks there until the 9th, returned home on the 10th, and fell ill on the next day. His wife fell ill 3 days after. The daughter-in-law remained well.

The stools, unfortunately, were not sent for bacteriological examination, so that bacteriologically the case cannot be proved to be one of Asiatic cholera, but the evidence in other respects can leave little doubt as to the nature of the case.

CASE E.—A full description of this case is unnecessary, for careful investigation failed to yield any evidence of infection, and although in the same house (of three rooms) 5 other persons aged from 3 months to 51 years (all members of the family) were living with the patient, no other case of illness occurred in the house. No case of the kind arose in the neighbourhood.

The patient had choleraic symptoms, and died in 4 to 6 days after their onset.

Post-mortem examination.—Small intestine intensely inflamed, contents hæmorrhagic and foetid, no typhoid lesions.

Bacteriological examination.—A large number of bacteria were separated by cultivation, the *B. coli communis* preponderating as in the other cases. There were numbers of large bacilli which liquefied gelatine very rapidly. *No comma bacilli* could be isolated.

SUMMARY.

We may say that these five cases constituted the cholera outbreak of 1893, in Manchester, as officially reported. Of these five cases, one, though reported as cholera, can hardly be considered to be a genuine case. In three of the remaining four cases cholera bacilli were found; in the fourth case the stools were not examined, but considering that this case (D) gave rise to Case C by infection, and in Case C the cholera bacilli were found, it is reasonable to surmise that they were present in Case D as well.

The four cases form two isolated groups, in each of which one person was infected; the one by living a few days in Grimsby, and the other by eating a large quantity of oysters from Grimsby, which was at that time infected with cholera.

Each of these cases, A and D, appears to have communicated the disease to one other person only, although others must certainly have been exposed to the chances of infection.¹

Of these 4 persons 2 recovered slowly, 1 died in 9 days, and 1 in less than 24 hours.

In one group the person who communicated the disease died, and the much older person to whom it was communicated recovered. In the second group the person who communicated the disease recovered, and the other died.

¹ Though the question of the mode of infection is undoubtedly interesting in connection with these cases, it is excluded by the scope of this paper.

The cholera bacillus was found in the two fatal cases, and in the stools of one of those who recovered. We have therefore, in this miniature outbreak, almost all the elements which have been recognised in the more serious epidemics. It is not our intention here to do more than point out how suitable the cases were for the study of the question of diagnosis, and we shall now revert to the question of

IV. THE VARIABILITY OF THE COMMA BACILLUS.

We have already shown that, in the first 24 hours, cultivations outside the body on nutrient gelatine gave at least four varieties of comma bacilli, recognisable by the rate at which they liquefied gelatine. Three of these varieties were studied and found to behave in every respect, but the rate of liquefaction of gelatine, like Koch's comma bacillus; each vibrio was provided with a flagellum at each end.

We will now describe three varieties—I, II, IV., all characterised by their behaviour when grown on gelatine, at about 21° C.:¹—

Type I. The most quickly liquefying, giving, in 48 hours, colonies over 1 mm. in diameter, were not at first studied; but, in the course of our further studies, similar colonies, giving the cholera-red reaction very well, were obtained (Plate XIII. Figs. 13, 15). They often began to liquefy gelatine at the end of 24 hours.

Type II. Quickly liquefying colonies.—At the end of 48 hours they measured about 0·7 mm. in diameter (Plate XIII. Fig. 11), and at the end of 24 hours (Plate XIII. Fig. 10) they were already larger than the next variety. No liquefaction was visible at the end of 24 hours. Liquefaction was advanced at the end of 48 hours (Plate XIII. Figs. 9, 10, 11, 12).

Type IV. Slowly liquefying colonies.—At the end of 48 hours they were about 0·26 mm. across; liquefaction beginning only when this size was reached (Plate XIII. Figs. 1, 2, 3, 4).

An intermediate form between I. and II. was found (Type III.), as well as a form which liquefied gelatine still more slowly than IV. (Plate XIII. Fig. 14, Type V.).

The question naturally arises, How could these rapidly liquefying organisms have been recognised before the days of the cholera-red reaction? and the answer, as it seems to us, must be, that such colonies were discarded as not being typical of the cholera bacillus. But a still more interesting question is suggested, Do not all these forms or sports indicate a tendency to variations, by which the parasitic form of the cholera bacillus might gradually pass into the saprophytic forms found in water and sewage?

Urged by this thought we continued the cultivation of the various types on various media, and found that they remained literally per-

¹ It was found more convenient to cultivate the bacilli at a temperature not above 21° C., in order to be able to keep under observation the slowly and quickly liquefying sports for at least 5 days.

manent, so long as they were passed from tube to tube in sufficiently large quantity. We have been able in this way to preserve types, separated by one of us last autumn, for 10 months. But at the beginning of the hot weather, in our plate cultures of the slowly liquefying variety, some colonies were seen liquefying the gelatine quite as rapidly as the most quickly liquefying variety obtained from the stools (Plate XIII. Fig. 15), and the reverse was true of the quickly liquefying type (Plate XIII. Fig. 14). The sport was permanent, therefore, only when all or a large number of the descendants of an organism of a certain type were kept together, and differences became evident as soon as they became separated on plate cultivations. The collateral influence was manifest in other properties than that of liquefying gelatine.

In connection with this we would also mention a phenomenon, which has undoubtedly attracted the attention of other observers: In an original plate cultivation, in which colonies of the cholera bacillus are abundant, these colonies appear smaller, and liquefy gelatine earlier than in the first or second dilution in which the colonies are sparser. In the last dilution some colonies liquefy gelatine very slowly indeed.

Milk sterilised in the autoclave, and at a temperature below 80°, when inoculated from ordinary cultivations of these spirilla did not become curdled; but when inoculation was performed from the surface layers of liquefied-gelatine cultures, 3 weeks old, the milk curdled in 3 days at 37° C. Fränkel (E.) had previously mentioned this.¹

By inoculating tubes of gelatine from the top layers of liquefied gelatine tube cultures we obtained forms (aërobic), which tended to liquefy the gelatine superficially (see Plate XIII. Fig. 9), and by inoculating from groups of colonies, lying at the bottom of the needle track, we obtained forms which thrived best along the needle track, and showed no disposition to liquefy the gelatine superficially (see Plate XIII. Fig. 1).

The outlines appended show the forms I., II., III., and IV., which were obtained from cultures originally isolated from the cases, by cultivation in the usual way, without any attempt being made to produce variation from the original form by selective plate cultures.

By selective cultivation from plate to plate of the quickest liquefying colonies of Type II., a sport, Type I., was obtained, which, as shown in Plate XIII. Fig. 13, gave rise to gelatine stab cultures somewhat resembling those of Finkler's vibrio. This sport seems to be pretty permanent, and it gives a distinct cholera-red reaction.

This sport was grown in bouillon of diminishing alkalinity and increasing acidity, until finally it grew in bouillon to which no alkali had been added. The cholera-red reaction in the peptone bouillon grew less and less marked as the alkalinity of the medium decreased, and finally we failed to obtain it in the acid cultures. At the same time, the vibrio assumed a coccoid form, and liquefied alkaline gelatine very slowly. On inoculation of the ordinary saline peptone water with

¹ *Deutsche med. Wchnschr.*, Leipzig, 1892, p. 884.

these cultures grown in acid media, the cholera-red reaction was again obtained within a few hours.

The specimen, III., also gave a sport, which liquefied gelatine as rapidly as I.; this, however, soon relapsed to the type of III.

An outline of an aberrant form of IV. is given (Plate XIII. Fig. 15); it is practically identical in appearance with the most rapidly liquefying of the original colonies, I., of the same age.

These last results, of course, are similar to those previously obtained by Hueppe,¹ who found that by altering the nature and reaction of the nutrient media, and by varying the supply of oxygen, he could, starting with a typical cholera vibrio, obtain various modifications in its chromo-genic properties and in its power of yielding the cholera-red reaction.

On investigation of the pathogenic properties of these vibrios in the summer of 1894 it was found that with II., one half of an agar culture grown for 20 hours at 37° was required to cause death in 2 days with the usual symptoms. The same amount of IV. produces only lowering of temperature. The same dose of III. was required to cause the death of a guinea-pig in 3 days.

We thus see that the more anærobic form IV. had become less virulent than the aerobic form II., and that the form III. had lost much of its virulence; these results are not comparable with those obtained by Hueppe, the methods used being different.

V. CONCLUSIONS.

1. With other observers we come to the conclusion that, by the application of a certain number of tests, it is pretty *easy to identify cholera vibrios* in the stools of patients suffering from a choleraic attack, when such vibrios are present.

2. *These vibrios, however, vary to a considerable extent* in their pathogenic, zymogenic, and chromogenic properties, and we have evidence that this is true, not only when they grow saprophytically (outside the body), but when they are obtained directly from the intestine of choleraic patients.

3. It seems to us that the *value of the statistical returns based on the bacteriological investigations of observers who ignored those variations* must be considered as giving us only part of the truth, because these observers must have considered many cases as free from cholera bacilli when these bacilli, though present, had abnormal characters.

4. On the other hand, we *are gradually led to recognise, as cases of cholera, many cases which formerly would not have been recognised as such.*

5. Bacteriological diagnosis has therefore not, up to the last two or three years, given us the means of obtaining perfectly comparable data.

¹ *Trans. Seventh Internat. Cong. Hygiene, etc.* vol. ii. p. 28, 1891.

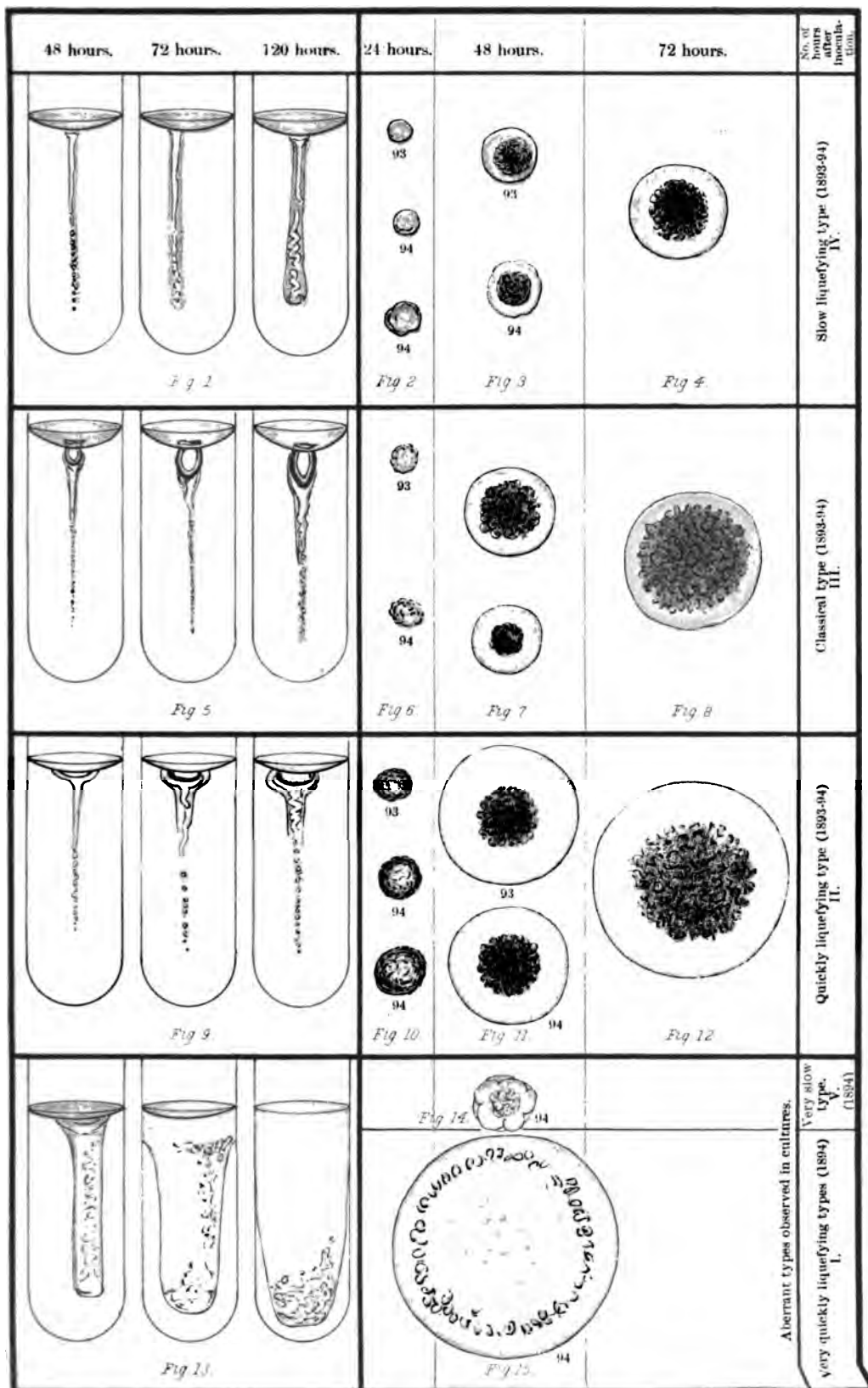




Fig. 16

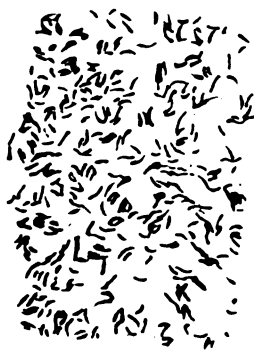


Fig. 17

DESCRIPTION OF PLATES.

PLATE XIII.

The sketches in this Plate are accurate outlines, copied from photographs, all taken under the same conditions.

The stab cultivations are of natural size.

The colonies from plate cultures are all magnified 30 times.

In the vertical column to the right, tube cultures of the four principal types are represented as they appeared after 48, 72, 120 hours' cultivation, at a temperature not exceeding 21° C. (In each case *the same tube* was photographed on the three occasions.)

In the vertical column to the left, microphotographs taken at the end of 24, 48, and 72 hours are reproduced (93 indicates the colonies which were photographed in 1898, *i.e.* immediately after they had been isolated from the intestinal contents, 94 indicates those colonies which have been obtained after several months' cultivation on various media).

Each horizontal subdivision corresponds to one of the types described in the paper, with the exception of the lowest, in which a small space has been given to the aberrant type V.

(From Drawings by S. Delépine.)

PLATE XIV.

FIG. 16.—Impure cultivation in peptone water. (20 hours after inoculation, with 3 loopfuls of stools from Case A. To show the proportion of *Bacillus coli* and cholera vibrio in the superficial layers. This culture gave a faint cholera-red reaction.)
× about 1000 diam.

FIG. 17.—A pure cultivation in bouillon, obtained from Case C. × about 1000 diam.

(These two photographs are by Mr. Andrew Pringle, from preparation by S. D.)

A CASE OF TYPHOID SEPTICÆMIA ASSOCIATED WITH FOCAL ABSCESES IN THE KIDNEYS, DUE TO THE TYPHOID BACILLUS.

By SIMON FLEXNER, M.D., *Associate in Pathology, Johns Hopkins
University.*

From the Pathological Laboratory of the Johns Hopkins University and Hospital.

(PLATE XV.)

THE distribution of the typhoid bacillus in the body in typhoid fever must be considered as still presenting interesting features. That the typhoid bacillus may be present in the blood of typhoid patients seems, in view of certain observations (Karlinski, Vincent), very probable, although the work of the earlier investigators (Rütimeyer, Almquist, Pasquale, Guarnieri, Neuhaus), who claimed to have cultivated the organism from the general blood and that of the rose spots during life, has, through the later results of Janowski, Stagnetto, Grawitz, Fränkel and Simmonds, and Sittmann, been discredited. It is, however, significant that the observations in which typhoid bacilli were believed to have been cultivated from the blood during life and again at autopsy, as in the case of Karlinski, date from a time when the means of distinguishing between the typhoid bacillus, and certain other bacterial varieties, much resembling this organism, were not so complete as now.

In individuals who have died of typhoid fever, provided death has occurred not too late in the course of the disease, it is usually possible to cultivate the typhoid bacillus from various organs, among which the mesenteric glands, spleen, liver, and kidneys (not to refer to the intestines) may be mentioned. The experience of this pathological laboratory indicates that the bile is particularly rich in the typhoid organisms, and the autopsy records of the Johns Hopkins Hospital contain many instances in which they have been isolated from this fluid in pure culture. Now that Quincke has directed attention to it, the bone marrow will doubtless be found to be a common habitat of the organism in the disease; and in several recent autopsies I have found the typhoid bacillus to occur in considerable numbers in this situation.

In abortion (Widal and Chantemesse, Hildebrand, Eberth, Ernst, Janizewski, Frascani) the typhoid bacillus has been found in a conclusive number of cases to have passed through the placental barrier, and to be present in the organs and even in the blood of the foetus.

The bacillus of typhoid fever is therefore distributed in such a way as to indicate that it has at some time during the disease been present in the blood current. Yet it must be remembered that it is not, unless most exceptionally, found in the blood outside of organs, even at autopsies. The rarity of the condition has suggested the advisability of recording a case in which there was a general infection of the body with the typhoid bacillus.

The case presents, in addition, another feature which is perhaps not without interest. The recent contributions to the study of the relation of the typhoid bacillus to local foci of inflammation have tended to show that in a variety of inflammatory processes the typhoid bacillus alone is concerned. This view, first advanced by A. Fränkel, has, as is well known, been vigorously contested by Baumgarten and E. Fränkel. The latter have contended that other organisms, particularly the pyogenic cocci, were present, along with the typhoid bacillus, and were overlooked or had died out at the time of the examination. The kidneys of the case to be reported here contained a number of small abscesses, in which only the typhoid bacillus was demonstrable.

The clinical course of the case was unusual. I am enabled through the kindness of Professor William Osler, to whose wards the patient had been admitted, to give an abstract of the clinical history.

CLINICAL SUMMARY.—*Illness of two weeks' duration before admission; moderate fever; enlarged spleen; rigidity of muscles of neck and of right arm; mental dulness and delirium; cutaneous hyperæsthesia and increase of the reflexes; small amount of albumen, with red blood corpuscles in the urine; no "dialysis" reaction; for 3 days before death normal temperature; parotitis.*

Susan B., æt. 18, coloured; admitted to the medical wards (Professor Osler), 23rd April 1894, complaining of headache, pains in the abdomen, and general weakness.

The patient is an only child; her father is living, her mother is dead, but from what disease could not be learned. There is a history of tuberculosis in the father's family.

She does not remember ever having had any of the diseases of childhood; was always strong and well; menstruated first at 17, and has been regular. Five weeks ago she began to have sick headaches, but without vomiting. She kept at work, though feeling wretched, until 2 weeks ago, when she began to have pains in different parts of the body, and weakness. She was restless and feverish, without appetite, slept very badly, and had diarrhoea. The patient was so dull and stupid that it was only with difficulty that a history could be obtained.

On admission at 5 P.M. her temperature was 103°·7; the pulse 120, soft and regular. She vomited a dark greenish material. That night she was restless, but the temperature did not rise above 103°·5. She was sponged, and whisky was administered. The urine obtained and examined on the evening of admission was slightly brownish in colour, acid; specific gravity,

1010; it contained a small amount of albumen, and microscopically showed leucocytes and red blood cells. No casts were seen; the diazo reaction was not present.

24th April.—The patient is a small, thin, poorly nourished girl, and looks as if she had been ill some time. She lies on her back with the eyes half closed, and the face has a dull, typhoidal expression. The temperature was $102^{\circ}6$ at 8 o'clock; the pulse was 116, regular. The abdomen is not distended, but is held rather tense. The spleen is palpable, fully 4 cm. below the costal margin. During the examination the patient complains much of tenderness everywhere. The examination of the lungs and heart showed nothing abnormal. The first sound at the apex was a little prolonged and dull. The patient had one stool since admission, which was small, firm, and somewhat clay-coloured.

25th April.—The patient had a better night; the temperature for the greater part of the last 24 hours has been below 102° . The urine is dark amber-coloured, acid in reaction; specific gravity, 1010; no diazo reaction; a small amount of albumen; contains leucocytes and a small number of red blood corpuscles. The patient had one movement to-day, in which there was a small faecal concretion, concentrically laminated. She had through the day a good deal of vomiting, and she took her nourishment—milk and albumen water—very badly.

26th April.—Patient has vomited less; temperature has not reached 102° ; no movement from the bowels; pulse is feeble, and ranges from 112 to 120. Only 400 c.c. of urine was collected; she passed some involuntarily. Towards the afternoon of the 26th it was noticed that the head was much retracted, and held very stiffly, and the right arm was in a condition of extension, somewhat rigid, and any attempt to flex it caused pain.

27th April.—The temperature to-day was lower, and at 6 A.M. reached $98^{\circ}9$, and the highest throughout the day was $100^{\circ}6$ at 6 P.M. She vomited repeatedly, talked incoherently, and moaned a great deal. She had one small stool at 10 P.M. She seemed very sensitive to the slightest touch. The retraction of the head and the rigidity of the arm persisted.

It did not seem possible to decide as to the nature of the case. Though it looked more like typhoid fever on her admission, the retraction of the head and rigidity of the neck muscles, the general sensitiveness and the rigid extension of the right arm suggested, very naturally, cerebro-spinal meningitis. The blood was carefully examined for malarial organisms, and cover-slips were stained for tubercle bacilli; the leucocytes numbered 6000 to the cubic millimetre. The urine was yellow, acid, distinctly cloudy, with heat and nitric acid; no diazo reaction. Microscopically there were many epithelial cells, chiefly from the bladder, some leucocytes, and a smaller number of blood cells. The sediment was collected and stained for tubercle bacilli with negative result.

28th April.—On account of the vomiting she has been fed, for the past 24 hours, by rectal injections. At the morning visit she was lying with the head thrown back, and resisted slightly any attempt to bend the neck. She replies to questions in a confused and hesitating manner. There is no photophobia; the pupils are equal and react to light. After being aroused, she lies with her eyes open, but with rather a staring expression. She moves the left arm readily, but the right is extended and motionless by her side. It is not so rigid to-day, though she winces and cries out when any attempts are made to flex it. The deep reflexes in the left arm are active; in the right they cannot be tested. The knee jerks are a little exaggerated, and there is well-marked ankle clonus on both sides. The abdomen is not swollen; not especially tender. As satisfactory an examination as possible was made of the uterus, but nothing abnormal could be detected. The temperature fell to

98°·2 this morning, and the highest registered through the day was 100°·1. The patient had one well-formed stool.

29th April.—The rigidity of the muscles of the neck is still marked, and the stiffness in the right arm persists. Mentally she seems somewhat clearer, says she has no headache, and puts out her tongue when asked. From 10 o'clock last night the temperature has been a little below 99°. She has been passing urine involuntarily.

30th April.—The temperature continues normal; was 98°·2 at 8 P.M. and 98°·4 at 8 this morning. The pulse is feebler, just 100, and not irregular. There is no strabismus. She moves the left arm about freely; the right arm shows slight clonic movements, and at intervals becomes quite rigid. The ankle clonus could not be obtained to-day. The abdomen is flat, and not more sensitive than in other parts.

May 1st.—Until 8 A.M. the temperature was about 99°. This morning it was noticed for the first time that there was a soft swelling in the left parotid region, which is not painful. The vomiting has been very obstinate. She retains the nutritive enemata. To-day the right arm is easily flexed, and can be moved in all directions. The heart sounds are, and have been, perfectly clear. There are few râles at the bases of the lungs, but there has been no diffuse bronchitis. The temperature rose a little through the day, and reached 100°·2; the pulse became more rapid and feeble, and she died at 5.30.

Autopsy 17 hours after death.

ANATOMICAL DIAGNOSIS.—*Typhoid fever (ileo-typhus and colo-typhus); hæmorrhagic enteritis; acute spleen tumour; multiple abscesses in the kidneys; parenchymatous degeneration of the liver and kidneys; purulent infiltration of the parotid gland; œdema of lungs and glottis.*

Body of a small, sparely built negro girl. Subcutaneous fat in small amount. Muscles bright red in colour.

Peritoneal cavity.—No excess of fluid. The intestines are distended.

Pleural cavities.—Both dry. The lungs are free from adhesions. Both are voluminous.

Pericardial cavity.—No excess of fluid, and both layers of the pericardium are smooth.

Heart.—Right auricle and ventricle contain dark and partly decolorised clots. In the left ventricle a small, partially decolorised clot. All the valves are normal.

Lungs (Right).—The upper and middle lobes are œdematous; the lower lobe is congested. The bronchi are filled with frothy serum. Vessels at base free. (Left).—Lower lobe congested; the upper lobe œdematous. Bronchi contain frothy serum; vessels at base free.

Liver.—Weight, 1700 grms. (about 60 oz.). It is soft, swollen, and the sharp edges are rounded. On section it is fatty in appearance, the lobular markings being obscure. The bile is thin, light-coloured, and contains a granular sediment.

Spleen.—Weight 420 grms. (about 14·1 oz.). The capsule is thin and distended. On section the substance is soft and pulpy; the follicles are visible and apparently not enlarged.

Mesenteric glands.—Swollen and congested.

Adrenal glands.—Apparently normal.

Kidneys.—They together weigh 320 grms. (about 11·3 oz.). (Left).—Large and pale. The capsule strips off easily, and beneath it, imbedded in the cortex, a number of small white nodules, larger than miliary tubercles, can be seen. Others are visible in the cut sections of the kidney. (Right).—In general the same as the left. In this kidney are several larger white areas, the largest of which is triangular in shape, the base of the triangle, measuring 4 mm., is situated at the surface. The capsule corresponding with this area contains small hæmorrhages.

Parotid gland.—On the left side it is swollen and infiltrated with pus.

Larynx.—The posterior epiglottic tissues and the aryteno-epiglottidean folds are infiltrated with serum, and present a gelatinous appearance.

Intestines.—The small intestine is bile-stained throughout. The mucous membrane is cloudy, and sticky mucus covers its surface. The patches of Peyer in the upper parts of the small intestine present the shaven-beard appearance. The first ulceration occurs in the ileum 200 c.c. above the valve. It is round, about the size of a 1-cent piece, and is superficial and clean. 160 cm. above the valve there is a large ulcer, involving the entire circumference of the gut; it is 10 cm. in width, is irregularly convoluted superficially, and the surface is necrotic and sloughy-looking. This ulceration apparently does not reach beneath the submucosa, but the whole thickness of the intestine is involved in the infiltration. On the serous surface small white points (lymphomata) can be seen. Between this ulceration and the ileo-cæcal valve, there are several smaller losses of substance. The lower end of the ileum is, together with the valve, inclosed in one larger ulceration of the same general appearance as the larger ulcer already described. Some of the solitary follicles of the cæcum are ulcerated, others are swollen, the centres of these often being necrotic. The ascending, transverse, and in part the descending colon, show the same conditions as the cæcum.

Beginning at the larger ulcer, 160 cm. above the valve, and continuing downwards to the latter, the mucous membrane is infiltrated with blood, the surfaces of the valvulæ conniventes being more affected than other places, except immediately about the ulcers. The swollen and ulcerated follicles in the large intestine are surrounded by a zone of hæmorrhage.

Fresh frozen sections of the heart muscle show it to be free from fat; sections of the liver and kidneys show parenchymatous and fatty degeneration of the epithelial elements of these organs.

Bacteriological examination.—Cover-slip preparations made at the time of the autopsy from *bile, spleen, and nodule in the kidneys*, showed many bacilli having the form and staining properties of the typhoid bacillus. This organism was found alone in these cover-slips. From the pus of the parotid gland, the cover-slip preparations showed many streptococci.

Cultures.—Agar-agar plates were made at the autopsy from the heart's blood (right auricle), lungs, kidneys, mesenteric glands, spleen, bile, liver, bone-marrow (femur), and parotid gland. These plates, kept at 37° C. for 48 hours, gave the following results:—

Bile.—Crowded with colonies, apparently all of the same kind. Proven to be a pure growth of the typhoid bacillus.

Spleen, mesenteric glands, and bone marrow, all contained many colonies of the typhoid bacillus.

Kidney.—Two cultures were made from this organ. The first was taken from one of the white nodules, the second from the intervening kidney substance. From the nodule many colonies developed, and they consisted entirely of the typhoid bacillus. The other plate contained fewer colonies, from which two kinds of organisms were separated, one agreeing with the typhoid bacillus, the other with the *Bacillus coli communis*.

Lungs.—From the congested portion of the right lower lobe three organisms were isolated—(1) *Streptococcus pyogenes*; (2) *Typhoid bacillus*; (3) *Bacillus coli communis*.

The streptococcus colonies predominated.

Heart's blood.—On this plate 20 to 30 colonies, all having the same features and proving to be the typhoid bacillus, developed.

Parotid gland.—The agar-agar plate was crowded with minute colonies of the *Streptococcus pyogenes*. Sections of the gland hardened in alcohol showed a pure streptococcus invasion along the ducts into the gland acini, associated

with an interstitial and parenchymatous infiltration of leucocytes, with polymorphous nuclei.

In studying the bacilli isolated from this case, special attention was paid to distinguishing between the typhoid and the colon bacillus when they occurred together (lungs and kidneys), and to proving that the organisms found in the heart's blood, kidney nodules, as well as in the other organs mentioned, were really typhoid bacilli.¹

The weight of opinion among bacteriologists at this time, is that the colon group of organisms can be sharply differentiated from the typhoid bacillus. However, it no longer suffices for distinguishing them to observe the colony growth upon agar gelatine plates, or the growth upon potato; neither does the possession of mobility and stainable flagella serve to distinguish the typhoid from the colon bacillus. On the other hand, the power of acid and indol-production, the property of setting up fermentative changes in sugar with the liberation of gas, and the coagulating influence upon milk, possessed by the colon group of organisms, separate it from the typhoid organism, which produces alkali, does not yield indol in cultures, is not capable of fermenting sugar, and has no coagulating effect upon milk.

The bacilli separated from this case were tested by these means, by observations of their growth upon different solid culture media, as well as by microscopical study; and it was found that in the heart's blood, spleen, bile, liver, mesenteric glands, bone-marrow, and abscesses of the kidneys, the typhoid bacillus was present in pure cultures.²

The number of observations at the present time referring to the

¹ The recent writers on the means of distinguishing the typhoid and colon bacilli regard the fermentation (Smith, Chantemesse and Vidal, Fuller, Dunbar, Welch, Valentini, Germano and Murea, Silvestrini), milk (Dunbar, Maloz, Valentini, Welch, Blochstein, Silvestrini), and indol (Péré, Ferrati), tests as sufficing to establish this distinction, and my own experience accords with that of these authors. Recently a new medium has been introduced by Wurtz for separating these two forms. It consists of lactose-gelatine coloured with an alkaline litmus tincture. The colony growth in this medium can, through the change in the reaction produced in it, be used for this purpose. The colon bacillus colonies are said to become pink, whereas the typhoid remain blue. In several tests recently made with this medium, agar being used in place of gelatine, I found that the typhoid produced sufficient acid when acting upon lactose to turn the medium slightly pink; but the acid reaction is less strongly marked than that caused by the colon bacillus. I have, moreover, had the opportunity of observing a variety of the typhoid organisms which, when planted in litmus milk, gives at first a typical reaction; that is, after 24 to 48 hours at 37° C., it causes a faint pink colour to appear. This colour persists for some days (5 or 6 in the thermostat), when it changes back to blue, the blue gradually increasing in intensity until after 10 days or 2 weeks, the alkaline reaction is very prominent. This fact agrees with Wurtz's observations on the alkaline (ammonia) producing power of the typhoid bacillus, as opposed to the acid production of the colon bacillus. Pfeiffer has, moreover, just pointed out that animals rendered immune from the typhoid bacillus, contain an anti-toxine in their blood which is bactericidal to the typhoid bacillus, and not to the colon group of organisms, and other forms which seem to be even more closely related to the typhoid bacillus.

² The typhoid bacillus has been found in local inflammations as follows:—In connection with bone giving rise to osteomyelitis and periostitis, by Ebermaier, Orlow, Valentini, Achalme, Chantemesse, Colzi, Melchoir, Dupraz, Buschke, Sultan; in abscesses of skin and

typhoid bacillus as the cause of post-typhoid inflammations is so large as to admit of little doubt of an etiological connection between the organism and the lesion process. The length of time during which this organism can remain in the body is not a little surprising. Among the first of the observations bearing on this point, is that of Welch, who found in a rabbit which had been inoculated with typhoid bacilli 4 months previously, and had recovered, and which later succumbed to another experiment, that the typhoid bacilli which had disappeared from all other situations were still present in considerable numbers in the bile of the animal. The longest period yet recorded in which the typhoid bacillus had been found in the body after recovery from a typhoid fever, is that reported by D. Buschke, in which, in a rib-abscess, they were present seven years after. The list of cases in which this organism has been found in local foci of suppuration is now very large, and includes most situations in the body. In the study of some of these it is not improbable that the strict bacteriological requirements, concerning, in the first place, the identity of the bacillus, and in the second the absence of other organisms, may not have been carried out. But a few carefully studied cases, in which the typhoid bacillus is proven to have been present alone, renders the doubtful ones more probable. In the study of the abscesses in the kidneys¹ in this case, this fact was kept in mind. Therefore, examinations were made—(1) of cover-slip preparations from the abscesses, (2) of cultures in agar-agar, etc., (3) sections from the kidney hardened in alcohol were stained in various ways, as will appear, for organisms, with the result that the typhoid bacillus was found alone in the lesions.

The abscesses can be separated into two groups, depending upon their sizes. The smaller ones are limited to the cortex of the kidney. Their shape, as found upon microscopical examination, is linear rather

muscle and periarticular abscesses, by Melchoir, Raymond, Rosin and Hirschel, and Swiezynski; in acute endocarditis, by Carbone, Vincent, Girode; in circumscribed peritonitis, by A. Fränkel, Lehmann; in cerebro-spinal meningitis, by Kamien, Honl, Hintze, Stühler, Mensi and Carbone, Vincent; in abscesses in the spleen and mesenteric glands, by Roux and Vinay, Lehman, Flexner; in purulent cholecystitis, Gilbert and Girode Chiari; in sero-fibrinous pleurisy and empyema, by Ferret, Loriga and Pensuti, Valentini, Spirig, Weintrand; in purulent strumitis, by Colzi, Honl, Jeansalme; in suppurative epididymitis, by Gilbert and Girode; and in suppurative orchitis, by Tavel; and in a suppurating lipoma of the knee by Sittman.

¹ The typhoid bacillus has been found in the kidney by the culture method in many instances, in which no lesions have been detected, and in association with lymphomata by several authors, viz., Koujojeff, Spirig, Karlinski. Faulhaber was able to grow from an anæmic infarction of the kidney, in a case of typhoid fever associated with fresh vegetations on the mitral valve, a large number of typhoid colonies. The lymphomata are described as usually microscopic in size, but they may reach a size sufficiently large to be seen with the unaided eye. They are composed of accumulations of round cells with single nuclei. The bacilli are present, so far as they can be seen, in small masses and groups, lying amid the cellular infiltrations. Koujojeff saw them in both capillaries and tubules, while Spirig could not clearly ascertain their situation. Neither discovered them in the glomeruli. Von Wunschheim has just published a report of 2 cases, in which abscesses in the kidneys were produced by the typhoid bacillus.

than round, the central zone richly infiltrated with pus cells, being surrounded by a wide but less thick, round-cell infiltration. Specimens stained for 2 hours in Loeffler's methylene blue, differentiated in 1-1000 acetic acid, dehydrated in absolute alcohol, and cleared in oil of cloves, show, under a low power,—objective No. 3, ocular No. 3,—large masses of bacteria; in a single section as many as six of these collections of bacteria may be visible. These masses of bacilli are entirely and promptly decolorised by Gram's method of staining, and no other organism could be found, after painstaking search, in specimens stained in this manner.

Toward the centre of such an area the cellular infiltration is so great as to obscure the structure of the tissues; and when the latter can be made out the staining is feeble, and in it many nuclear fragments are to be seen. The infiltrating cells vary in form, some of them possessing single and others compound (polymorphous) nuclei. At the edge of the infiltration it can be readily seen that many of the cells are intertubular. These partake as a rule, although not exclusively, of the character of small round cells. Some of the tubules are dilated and filled with polyform leucocytes. The masses of bacteria are often so surrounded by cells, that their location is not always to be made out with certainty. But they appear at times to be intratubular; besides the large masses described, smaller foci of bacterial accumulation are to be seen among the cells.

The larger foci involve the medullary as well as the cortical part of the organ. From the surface the infiltration extends in somewhat irregular parallel lines for a considerable depth into the pyramids. In such an area the "nuclei" of the cells are to a large extent of the polyform (polynuclear) variety. However, between the tubules, at a little distance from the thickest infiltration, there are many small cells with spherical nuclei present. In the central foci of leucocytic infiltration the kidney tubules are dilated and filled with pus cells. Tubules of the convoluted type are often distended to more than twice their normal size, and the straight tubules of the medullary rays are equally affected. Following the tubules downward into the pyramid, the intratubular exudate is very common, while the intertubular is more scanty.

The bacteria are in large masses and in smaller aggregations, and may now be seen to be distinctly inside of the tubules, and surrounded by pus cells. Indeed, some cells appear to have taken up the bacteria. The forms of the bacteria are readily made out in the smaller accumulations. They consist of short plump rods, with rounded ends, which in the alkaline methylene-blue fluid show an irregularity of staining. The larger masses can be easily seen with a No. 3 objective (ocular No. 3), whereas the smaller groups require the aid of the one-tenth in. oil immersion to bring them to light. A few small groups of bacteria were observed between the tubules, probably intravascular (Plate XV. Fig. 1).

An interesting feature was observed in several glomeruli in infil-

trated areas. Masses of thrombi of bacteria were seen in the afferent vessels, and similar bacilli were present in the glomerular capillaries, and had passed through these, as they were found again in numbers in the capillary space. The epithelium lining the capsule of Bowman was in such cases entirely degenerated (Plate XV. Fig. 2).

The epithelium of the cortical tubules, particularly of the convoluted portions, is swollen, granular, and often degenerated. Those tubules, which are adjacent to, or within, the areas of cellular infiltration, show at times an absence of stainable nuclei in the lining cells. The form of epithelial degeneration, which is seen most frequently, is, however, of the hyaline variety, and the hyaline material, which is highly refractive, can be seen to run together to form actual tube casts. Those tubules, in which many pus cells occur, have no demonstrable epithelium, or are lined by low, compressed cells, often still possessing stainable nuclei.

So far as these foci are concerned, the process is not distinguishable from acute suppuration, although the tissues show no particular tendency to soften and break down.

It should be mentioned that in a few places there are considerable masses of bacteria inside of tubules, showing less reaction around them, on the part of the tissues, than that described. Such appearances are uncommon in the cortex, but in the pyramids, in the collecting tubules, masses of bacilli are embedded in pus cells, with little or no surrounding intertubular infiltration.

There can be no doubt that the bacilli gained entrance into the tubules through the glomerular capillaries. This is shown not only by the appearances already described, but also by the almost complete absence of the bacilli in the intertubular tissue. It would appear that most of the pus cells seen in the pyramidal tubules also come from places higher up in the kidney.

The size of the masses of bacteria in some of the pyramidal tubules suggests that an increase must have taken place after these parts were reached. In a few instances a tubule, usually in the papillary portion of the pyramid, has been completely filled by a growth of the bacilli, and around these there may be no reaction whatever, so that a post-mortem increase is not to be excluded.¹

In considering the features presented by this case in the light of

¹ A few cases of especial interest have been reported in which the typhoid bacillus has been widely distributed in the body in association with definite lesions, or in which this organism has been isolated from the organs at autopsy with very slight or even entire absence of lesions in the intestine indicative of typhoid fever. It is at this time becoming to be very circumspect in the interpretation placed upon these cases, unless the most rigid requirements as to the identity of the organism are carried out. Hence in the cases reported by Banti and Karlinski of a typical typhoid fever, where no definite lesions of typhoid fever existed, and yet the organisms were obtained from the blood during life, and the organs after death, it may be questioned whether they are to be accepted as conclusive. But the same is not true of the case reported by Du Cazal in which, upon the closest inspection, no lesion could be found in the intestine. Yet typhoid bacilli which behaved in a typical manner upon various culture media, and did not ferment lactose nor redden Wurtz's

the literature bearing on the typhoid bacillus, it would seem as if it were necessary to regard this organism as being concerned in a variety of pathological conditions. Just as a larger and fuller acquaintance with the properties of the *Diplococcus pneumoniae* has shown it to be at one time able to cause as definite a pathological process as acute lobar pneumonia, at another abscess formation, and at still another a general infection of the body, so the typhoid bacillus seems capable of producing on the one hand the typical lesions and classical features of typhoid fever, and on the other of localised foci of inflammation and suppuration, and finally a genuine typhoid septicæmia.

It would be interesting if not profitable to inquire into the peculiar conditions which permit at one time the invasion of the blood by this organism, whereas, as a rule, it does not find in it a suitable medium for development. The fact is too well known to need repetition, that normal human blood serum quickly kills many typhoid bacilli. It is not improbable, therefore, that in such instances the germicidal power of the blood has been either annihilated or diminished, and it may be well to ask whether, as in the experiments of Vincent and Chantemesse and Widal with the streptococcus and its soluble products, or of Sanarelli with the products of the *Bacillus coli communis* and the *Bacillus proteus*, the general infection might not be explained by the development of a concomitant infection, in this case a streptococcus parotitis. Vincent has shown, moreover, that in human beings a streptococcus invasion of the body makes the prognosis very grave, even when it occurs towards the close of the disease, and after the typhoid bacilli have almost disappeared from the system. Among his cases is one that was complicated by an otitis media of streptococcus origin, in which on the fifteenth day there was augmentation of the fever, and an inverse type of temperature. Seven days later death occurred. The autopsy showed a general streptococcus and typhoid infection, the former organisms predominating in the heart's blood. But as opposed to such a supposition as the above, are the many instances of suppuration due to the pyogenic cocci, in the course of typhoid fever, in which there is an absence of evidence pointing to an invasion of the blood by the typhoid organisms. However, it may be well to study more particularly the relation of the typhoid bacillus to the blood in human beings in this class of cases.

agar, were isolated from the much enlarged spleen. The symptoms during life were those of typhoid fever, and at the autopsy the mesenteric glands, spleen, and kidneys were swollen and congested. The case of Vincent contained a number of superficial ulcers in the ileum, and a deeper one just at the valve. The spleen was swollen, and in it was an abscess the size of a walnut, containing thick, viscous, white pus; the mitral valve contained several fresh vegetations; there was a hæmorrhagic infiltrated plaque, the size of a 5-cent piece at the summit of the left ascending parietal convolution, and from all these typhoid bacilli in numbers were obtained in pure culture. They were also grown from other organs and the heart's blood. The bacilli were decolorised by Gram's method, and grew like typical typhoid bacilli in bouillon, Vincent's (carbolic acid) bouillon, and upon agar-agar.

DESCRIPTION OF PLATE XV.

FIG. 1.—Represents a glomerulus, highly magnified, which is surrounded with pus cells, and in which typhoid bacilli are seen in the entering vessels, in the capillaries, and also free in the lumen of the capsule of Bowman.

FIG. 2.—A portion of the cortex is drawn under a low magnifying power (Ziess, Obj. D, Oc. 3) to show the extent and position of the exudate, and the relation of the clumps of typhoid bacilli to the infiltration. The deep blue clumps are masses of typhoid bacilli; they are seen in the tubules embedded in pus corpuscles. The small blue, somewhat irregular nuclei represent the nuclei of pus corpuscles.

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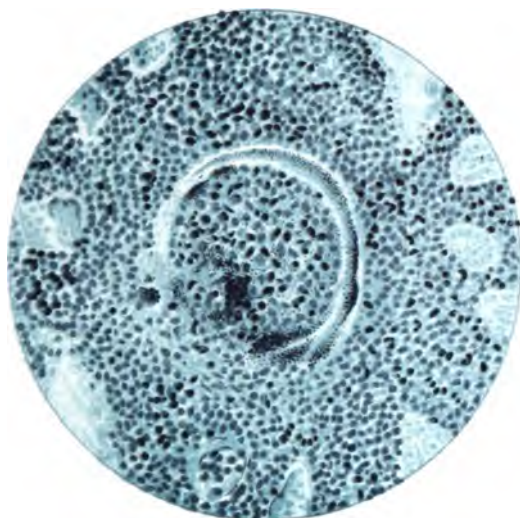


Fig. 1

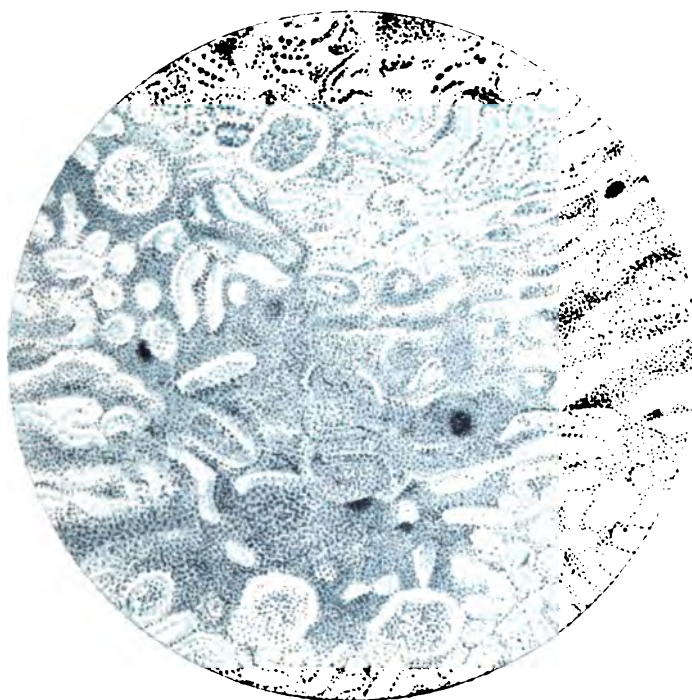


Fig. 2

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EXPERIMENTS WITH THE PNEUMOCOCCUS, WITH ESPECIAL REFERENCE TO IMMUNITY.¹

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IN opening this discussion, I propose to touch lightly upon all those points which I have not studied, especially, myself; but I shall treat in detail that part of the subject in which I have had the greatest experience.

I hope that by giving a brief outline of the whole matter I shall facilitate the discussion which will follow this paper:—

HISTORY.

With regard to the history of the subject I shall not detain you longer than to point out the confusion that arose at the time when the pathology of the disease was first studied. Friedländer described diplococci as occurring constantly in the exudation from pneumonic lungs. These diplococci were characterised by possessing a capsule; and on making cultivations on gelatine, a capsulated diplococcus grew, and was considered by Friedländer to be the cause of the disease. For some time Friedländer's pneumococcus was accepted as the cause of pneumonia. Further investigations, especially those of Fränkel, Talamon, and Weichselbaum, showed that the diplococcus cultivated by Friedländer was only present in a few cases of pneumonia, and that, in fact, it was only of accidental occurrence. It differed also in morphology from the diplococcus found microscopically in pneumonic lungs, being more elongated, and resembling a bacillus rather than a coccus. Talamon and Fränkel, however, were enabled to cultivate the true pneumococcus in broth and on agar, and they found that it differed entirely from Friedländer's pneumococcus both in virulence and in its mode of growth. It is with this pneumococcus that we have to deal.

¹ Read before the Pathological Society of London, in opening the discussion on the Pneumococcus, and published with the permission of the Council of the Society.

THE PNEUMOCOCCUS AS THE CAUSE OF DISEASE IN THE
HUMAN SUBJECT.

There is a vast mass of evidence to show that the pneumococcus is the cause of acute lobar or croupous pneumonia. It is also responsible for some cases of lobular pneumonia, but the majority of the cases of this latter disease are caused by other bacteria, notably the streptococci.

But the pathogenic rôle of the pneumococcus in the human body is not limited to the production of pneumonia. It is the cause of many cases of pleurisy and empyema, meningitis, and otitis, occurring quite independently of pneumonia; and is also the cause of ulcerative endocarditis, and of various suppurative affections of the joints and other tissues following upon an attack of pneumonia.

Last year, in a paper read before the Royal Medico-Chirurgical Society, I brought forward evidence to show that the constitutional symptoms of pleurisy and empyema, when caused by the pneumococcus, are exactly similar to those of pneumonia; and I have no doubt that they are due to the same cause, *i.e.*, the absorption of the toxins produced by the pneumococcus. In other cases, such as meningitis, the general symptoms are masked by the local effects of the inflammation. This is quite in accord with what occurs in other microbial diseases, such as influenza and malaria.

With regard to the *distribution of the cocci* in cases of lobar pneumonia, I have had but little experience. They, however, are found most abundantly in the affected lung, and are generally absent from the viscera; but a number of cases have been recorded in which the cocci have been found in small numbers, in the spleen, kidneys, and blood.

THE OCCURRENCE OF THE PNEUMOCOCCUS IN THE SECRETIONS FROM
HEALTHY INDIVIDUALS.

The pneumococcus has frequently been found in the secretions from the mouth, nose, and bronchi of healthy individuals, without producing any symptoms. Indeed, it was first discovered by Sternberg, by inoculating rabbits with the saliva of healthy individuals.

Now this is no argument against the pathogenic rôle of the pneumococcus. The same applies to the diphtheria bacillus, which has frequently been found in the throats of healthy individuals without producing any injurious effects. In a note by Dr. Hopwood and myself, in a recent number of the *British Medical Journal*, we mentioned a case where virulent diphtheria bacilli were constantly present in the throat of a healthy individual for so long a period as six weeks.

CULTIVATIONS OF THE PNEUMOCOCCUS.

The pneumococcus is a difficult micro-organism to work with, on account of the variability in its virulence, and of a number of peculiarities in the manner of its growth.

In the course of my investigations I have inoculated some 200 animals (some of these experiments have been made abroad), and have made innumerable cultivations upon different media, so that I can claim to have some acquaintance with its life history.

To obtain cultivations from the human subject the best method is to inoculate mice or rabbits with material containing the cocci. The animal dies after a variable time, and from its blood cultivations can be obtained.

CONDITIONS OF GROWTH OF THE PNEUMOCOCCUS.

The growth depends upon the temperature, and upon the nature of the medium in which the cultivation is made. As a rule, no growth occurs at temperatures much below that of the human body; although varieties have been described which grow at a temperature of 20° C.

As to the medium, I consider the reaction to be the most important factor; unless the medium is distinctly alkaline, no growth takes place, but here again varieties have been described which will grow in an acid medium.

The extreme rapidity with which cultivations die out has been noted by all those who have worked with the pneumococcus. Agar cultivations generally die within a week, and broth cultivations within 2 or 3 days. I have often found both cultivations dead at the end of 2 days. Many investigators have, from time to time, recorded exceptional instances where the cultivations have retained their vitality for a long period.

In one instance, I injected 1 c.c. of a broth-cultivation 23 days old into the peritoneal cavity of a rabbit. Death ensued in 4 days, and the blood was found to be crowded with pneumococci. In another instance, I examined a cultivation upon the surface of agar, and found it alive 64 days after it had been planted.

Emmerich, who has made some interesting observations upon this question, has come to the conclusion that the pneumococcus forms spores. He made the following observations:—A cultivation was made in half a litre of broth, and after incubation for 2 days was placed in the dark, at the room temperature, for 2 months. The whole of the sediment from the cultivation was then transferred to a tube of broth and placed in the incubator. A growth of pneumococci occurred in the tube. It is necessary to take the whole of the sediment, because only a few spores are formed, and a small quantity of the sediment may not contain any.

Arkharow mentions that in old cultivations, among a large number of dead cocci, a few are sometimes met with which stain well. I have frequently made the same observations, and I think it probable that these cocci which stain well represent the spore forms.

The short life of the pneumococcus under ordinary circumstances renders investigation difficult; for, unless fresh inoculations are constantly made, the cultivations die out, and fresh cultivation must be obtained from the human subject.

Foa and Arkharow have got over this difficulty by preserving the blood of infected rabbits in sealed tubes in the dark.

I have adopted this method, and have been able to keep the cocci alive, and virulent for as long a period as 3 weeks; but after this time they usually die.

The method is not altogether satisfactory; for, owing to clotting, there may be a difficulty in filling and emptying the tubes. I consequently searched for a better method of preserving the vitality of the cultivation. This I have attained by cultivating upon the surface of agar covered with a thin layer of blood, according to the method of Pfeiffer for the influenza bacillus.

I adopted this method, because I had observed that cultivations on agar made from the blood of infected animals retained their vitality longer, when a large quantity of the blood was transferred to the tubes. The tubes are easily prepared by spreading, under aseptic precautions, the blood from an animal recently killed. They are placed in the incubator for 24 hours to see that they are sterile, and are then ready for use.

On this medium the pneumococcus grows well, and retains its vitality and virulence for as long a time as 50 days. I have been using this method during the whole of last year, and have found it very satisfactory.

For the purpose of inoculation I make a cultivation in broth, and use this for injection.

I will quote an example to show how long the virulence is preserved. A broth cultivation was made from a blood-agar tube 40 days old. One c.c. of this was injected into the peritoneal cavity of a rabbit. The animal died in 14 hours from pneumococcal septicæmia.

By this procedure experiments with the pneumococcus are much facilitated; for there is no danger of the cultivation dying out at inconvenient times.

I find that E. Fränkel and Reiche, in the *Zeitschrift für klinische Med.* for 1894, have quite independently made the same observations, and were led to use this method of cultivation by the same course of reasoning.

Defibrinated rabbits' blood is also a good medium for the growth of the pneumococcus. I have been using this medium for the preparation of toxins, and find that the pneumococcus retains its vitality as long as 56 days.

MORPHOLOGY OF THE PNEUMOCOCCUS.

The morphology of the pneumococcus varies according to different conditions of growth. The usual form is that of a diplococcus. The prevailing shape of the individual cocci is lanceolate; but they are sometimes rounded, or oval, or even rod-shaped. In size they vary very much. Straight chains of 3 or 4 cocci are common, and long, twisted chains containing 40 or 50 cocci, are met with.

In the blood of animals, diplococci are almost exclusively found, and they are generally provided with a capsule. The capsules vary in size and distinctness; and in some cases I have failed to discover any at all.

In cultivations on artificial media the capsule is usually absent.

In peritoneal exudations, the individual cocci are often elongated so as to look almost like bacilli.

In broth cultivations short chains are common, and in cultivations made in broth from blood-agar tubes, the chains are often long, and no diplococci are to be found.

I have met with the longest chains in cultivations in the blood serum of immune rabbits.

I have always found these streptococci forms to be converted into diplococci in the blood of inoculated rabbits.

While discussing the morphology, I may mention that there is a bacillus producing septicæmia in rabbits, which, under certain conditions of growth, bears a resemblance to the pneumococcus.

At one time, after inoculating rabbits with the pneumococcus, I found, in the blood, bacteria differing from the pneumococcus in character.

I thought at first that I had introduced some impurity, but was at a loss to discover how. The matter was soon cleared up by the death of several rabbits in the stock cage from a form of septicæmia. The blood contained bacteria belonging to the group of *Septicæmia hæmorrhagica*, which often appear under the form of diplococci.

I have no doubt that the peculiar bodies described by Arkharow, as occurring in the blood after inoculating immune rabbits with the pneumococcus, and considered by him to be modified pneumococci, were due to an epidemic of septicæmia hæmorrhagica. Metchnikoff, in a footnote to Issaef's paper, makes a similar remark about Arkharow's work.

PATHOGENIC EFFECTS IN ANIMALS.

Many animals are susceptible to inoculation with the pneumococcus; others enjoy immunity. The most susceptible animals are mice and rabbits. *Guinea-pigs* are much less susceptible, but die when inoculated with virulent cultures in the peritoneal cavity. In the inoculations I have made, the animals died in from 1-7 days. At the post-mortem there was extensive peritonitis, with many cocci in the exudation; the blood in the cases dying early was crowded with cocci, but in the pro-

longed cases only a few were present. *Mice* die in 2 or 3 days after subcutaneous inoculation, and the blood is crowded with cocci.

Dogs are said to be more susceptible to subcutaneous than to intravenous or intraperitoneal inoculation.

Pigeons and fowls appear to be immune. I have made several attempts to infect these birds, but have always failed.

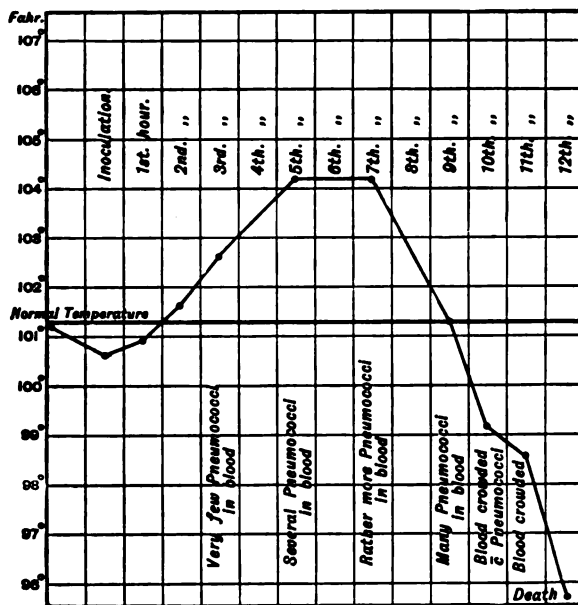
Rabbits are very susceptible, and as my experiments have chiefly been conducted on these animals, I shall quote my observations in detail.

EFFECTS ON RABBITS—INTRAPERITONEAL INJECTION.

The most satisfactory method of inoculation is to inject either the blood of an infected animal or a broth cultivation (1 day old) into the peritoneal cavity. I usually inject 1 c.c. of a broth cultivation or .5 c.c. of blood. The advantage of this method is that the animal almost always either dies within 2 days, or recovers. In only 1 case, among some hundred, of inoculation with cultures of different virulence, has death occurred as late as 3 days; and in one case as late as 4 days. This rule also holds good for immunised or partially immunised animals. If protective serum is injected with the cultivation into the peritoneal cavity, then death may be prolonged as late as 19 days.

With the cultivations I am now using, death occurs in 12–16 hours.

The most marked symptoms produced by a cultivation of this virulence are fever and dyspnoea.



The course of the fever is shown in the above chart. (The temperature is always taken in the rectum, and this is an important point,

for, as Dr. Hale White and I have shown, the temperature of the groin of a rabbit is always higher than that of the rectum.)

Immediately after the inoculation the temperature begins to rise, and reaches its maximum of 104° F. in about 5 hours. The temperature remains at the maximum for 2 or 3 hours, and then begins quickly to fall. For 2 or 3 hours before death it is subnormal.

Respirations.—The respirations increase in rapidity with the temperature, and by the time the latter is at its maximum they begin to be laboured. As the temperature falls, the dyspnoea becomes more marked, the head moving with each respiration.

GENERAL CONDITION.

The animal refuses food, but does not suffer any pain, and it is not until the temperature begins to fall that it appears at all ill. It then becomes quiet and crouches up in a corner of the cage, and remains in this condition until it dies. Death is often preceded by convulsions.

THE INVASION OF THE BLOOD BY COCCI.

I have made a number of observations upon the presence of the cocci in the blood at different times after inoculation. Either the animals were killed at different stages, or a drop of blood from the ear was examined during life.

At the end of 3 hours a few cocci are found in the general circulation, and the number increases as the temperature rises. It is only, however, when the temperature begins to fall that any large numbers are found. During the last 2 or 3 hours of life the blood is crowded with cocci. I cannot, therefore, agree with those who consider that the large number of cocci found in the blood at the autopsy is due to a multiplication occurring after death.

SUBCUTANEOUS INOCULATION.

The result of subcutaneous inoculation depends upon the virulence of the cultivation and the quantity injected. If the cultivation is very virulent, and as much as .5 c.c. of blood or 1 c.c. of broth is injected, death occurs within 12–16 hours without any local lesion, and with the same symptoms as occur after intraperitoneal inoculation.

If a smaller quantity is injected, or if the cultivation is less virulent, death occurs at a later period, generally in from 3–6 days; sometimes later; and in one of my experiments as late as 36 days after inoculation.

When death occurs at a late period, the most marked symptom is emaciation; the weight in one case falling from 3000 to 1780 grms. In 2 cases where death occurred, with marked emaciation, after a long

period, changes were found in the kidneys, and no cocci could be found in the organs or blood. Cases of death from marasmus, some time after inoculation, have been recorded by other observers.

LOCAL REACTION.

The extent and nature of the local reaction depend upon the duration of life. If death occurs within 12–16 hours there is usually œdema at the seat of inoculation; but in some cases I have found no local reaction whatever.

When death occurs in 2 or 3 days there is extensive œdema; in 1 case an enormous œdema of the head and face followed upon inoculation at the base of the ear.

When death occurs between the fourth and tenth days a hard swelling forms at the point of inoculation. The swelling consists of a fibrinous, or of a mixed fibrinous and serous exudation.

When death occurs late, or if the animal is partially immunised, and recovers, an abscess forms. The skin over the abscess generally sloughs and gives exit to a thick creamy pus.

These lesions are of interest, as showing that the same micro-organism may give rise to various forms of inflammation—serous, fibrinous, or purulent; and we can thus understand how the pneumococcus may produce in the human subject such conditions as fibrinous pneumonia, acute œdema of the lung, fibrinous pleurisy, empyema, otitis, etc.

POST-MORTEM APPEARANCES.

Peritonitis is usually present after intraperitoneal inoculation, but with very virulent cultivations the peritoneum may appear quite healthy. It is not uncommon to find peritonitis after inoculation in the subcutaneous tissue of the abdomen.

The peritoneum may be covered with a fairly thick layer of fibrin, or there may be only a few shreds of fibrin between the coils of the intestines.

In most cases no fluid is found in the peritoneal cavity, but sometimes a clear or turbid fluid is found.

The *intestines*, when there is peritonitis, are congested; and now and then hæmorrhages are to be seen in the mesentery or subperitoneal tissue.

The *lungs* are often congested, but frequently appear quite healthy.

Changes are not usually seen in the *liver* or *kidneys*.

The *spleen* is either quite normal in size and consistency, or is enlarged, and then may be abnormally soft or hard.

The *local lesions* at the seat of inoculation have already been described.

Edema in the anterior mediastinum is not uncommon.

The *blood* is usually clotted if the post-mortem examination is made a few hours after death, and in any case has always readily clotted on removal from the heart. It is almost always distinctly alkaline. Although I have often made inoculations with a pneumococcus quite as virulent as the one used by Issaef, I cannot confirm his observations with regard to the acidity of the blood, and the loss of power of clotting.

The *number of cocci* in the blood depends upon the duration of the disease and the local lesion. When death is the result of intraperitoneal inoculation, the blood is almost invariably crowded with cocci. The same is the case when death occurs in 3-4 days after subcutaneous inoculation, and there is only œdema at the spot of inoculation.

If death occurs at a late period, there is a fibrinous exudation, or an abscess at the spot of inoculation, and then the blood contains but few cocci, or none at all. A large number of cocci are usually found in the local lesion.

As *unusual lesions* I may mention *pneumonia* and *pleurisy*.

In 2 cases I found *pneumonia*. Both occurred after intraperitoneal inoculation, followed by death in 2 days. In 1 case the whole of the upper lobe of one lung was solid, and there were several patches of lobular pneumonia in the lower lobe. Microscopical examination showed that the alveoli were filled with cells. There was thick fibrin over the pleura. In the other case there was a patch of lobular pneumonia and pleurisy.

Pleurisy without pneumonia was observed in 3 cases.

In 1 of these cases the animal lived for 11 days after subcutaneous inoculation, and there was thick fibrin in the peritoneum, as well as on both pleuræ. In all the cases, when there was pneumonia or pleurisy, the heart blood only contained a few pneumococci. Several investigators have quoted cases where they have produced typical fibrinous pneumonia by inhalation experiments, or by intratracheal injections in dogs and rabbits.

Nephritis.—In two instances there was a parenchymatous nephritis. In both cases death occurred at a late period: in 1 case after 36 days, with much emaciation. The kidneys were speckled with yellow and white spots on the cortex, and microscopically fatty changes were found in the epithelium. No pneumococci were found in the blood or organs.

The observations of Fränkel and Reiche are interesting in connection with nephritis. In a number of cases of pneumonia in the human subject, they found changes in the epithelium of the kidneys, and from the juice of these organs they were able to obtain cultivations of the pneumococcus.

VIRULENCE OF THE PNEUMOCOCCUS.

The virulence of the pneumococcus varies, and is dependent upon a number of circumstances. In making comparative experiments, the quantity injected and the age of the cultivation must be taken into

account. The method of inoculation, whether intraperitoneal or subcutaneous, and the weight and age of the animal are important factors.

The direct injection of material from the human subject is no test of the original virulence of the cocci, because we cannot say how many living cocci are present in the material injected.

Kruse and Pansini have made extensive observations with pneumococci derived from different sources, and describe a large number of varieties differing from one another in virulence and morphology. They do not, however, consider these varieties to represent constant types. I am convinced that the virulence cannot be foretold from the morphological appearance. I have frequently found cultivations, consisting entirely of streptococci, virulent enough to kill rabbits in 12 hours when injected in doses of 1 c.c. into the peritoneal cavity.

VARIETIES OF PNEUMOCOCCUS.

There is, however, evidence to show that distinct varieties of the pneumococcus exist.

Fowitzky has separated a variety which produces a brick-red pigment, and which constantly produces pneumonia in rabbits on inhalation.

Foa describes two distinct varieties, both occurring in pneumonic sputum.

The one variety he calls the *Pneumococcus*, and the other the *Meningococcus*, because it is frequently met with in meningitis.

The *Meningococcus* or septic variety, after subcutaneous inoculation, causes death in rabbits in 3 days without local reaction. The spleen is hard and firm, and the blood full of cocci.

The *Pneumococcus*, or toxic variety, after subcutaneous inoculation, causes death in 24 hours. There is much œdema at the seat of inoculation, the spleen is soft, and the blood contains but few cocci.

I have made experiments with pneumococci from various sources, but have never succeeded in obtaining distinct varieties existing as constant types.

As far as the spleen is concerned, I have found it both hard and soft; and the inoculation with the blood of a rabbit dying with a hard spleen has frequently produced a soft spleen in another rabbit, and *vice versa*. In 1 case the spleen was four or five times its normal size, and remarkably hard, yet another rabbit inoculated with the blood of this one died with a soft spleen.

The nearest approach to the "toxic variety" I have met with was the following:—

A rabbit was inoculated in the subcutaneous tissue, and died in 24 hours. There was enormous œdema at the seat of inoculation, and the spleen was soft; and the blood, though containing many cocci, was not crowded with them, as is so often the case.

Now, the cultivation from this rabbit produced various types of the

disease in other rabbits; and again, the cultivation, from which this rabbit had been inoculated, had also produced different types of the disease, according to the quantity introduced and the mode of inoculation. As far as my own experience goes, the varieties of the pneumococcus have only differed in virulence, and can be converted into one another by repeated passages. In order to obtain a *type of constant virulence*, I have adopted the method of Issaef of frequent passages through the peritoneal cavity of rabbits, and in my later experiments I have used a type which appears to be constant: 1 c.c. of a broth cultivation, or .25 c.c. blood, injected into the peritoneal cavity of a rabbit, causes death in 12 hours, while .5 c.c. of the blood injected subcutaneously kills in about 14 hours.

I have found too, like Issaef, that sometimes, after repeated passages through rabbits, the virulence has become lessened; but that it has been regained by the passage through the body of a guinea-pig.

TOXINES.

With regard to the toxines produced by the pneumococcus, I propose to leave what I have to say upon the subject to a subsequent occasion.

PRODUCTION OF IMMUNITY.

Various methods have been adopted for obtaining immunity to the pneumococcus. All are based upon general principles, and consist in inoculating with attenuated cultivations or in injecting with chemical products treated in various ways. The method recommended by G. and F. Klemperer is the following: Recent broth cultivations are heated to 60° C. for 1 or 2 hours, and are used for injection. According to these authors, immunity is conferred at the end of 14 days, after subcutaneous injection of 24 c.c., and in 3-4 days after intravenous injection of 8-12 c.c. I have injected into the circulation of 14 rabbits, cultivations treated in this manner, in doses of 12-15 c.c., and subsequently the rabbits were inoculated with virulent cultivations at different periods after the injection, in order to see when immunity occurred, and how long it lasted.

The result showed that immunity was not conferred until after the twentieth day; that it was present between the twenty-fourth and fifty-first day, and then disappeared.

The rabbits inoculated on the fifth, ninth, sixteenth, and twentieth days died; those inoculated on the twenty-fourth, twenty-fifth, thirty-second, forty-third, and fifty-first lived; and those inoculated on the sixty-fourth, eighty-second, and eighty-fifth day died. Two of the rabbits died after the injection with the heated cultivation, one on the seventh, and the other on the tenth day. They were both much emaciated, but the organs appeared healthy, and no bacteria were found in the blood or organs.

Emmerich produces immunity by injecting into the veins very diluted cultivations, and Foà uses a glycerine extract of the blood of infected animals.

I have had no experience with these methods.

Several investigators have produced immunity by inoculation with *attenuated cultivations*. This method is unsatisfactory, because so many animals die during the process.

In the course of other experiments, I have in a few cases produced immunity in this way. For instance, a rabbit was inoculated subcutaneously with a small quantity of an agar cultivation, 4 days old. An abscess formed at the spot of inoculation, from which the animal recovered. At the end of 76 days it was inoculated with a virulent cultivation, and was not affected.

I have also produced immunity by the inoculation of *filtered cultivations*. For instance, 20 c.c. of a filtered cultivation in defibrinated blood was injected into the subcutaneous tissue of a rabbit; 21 days later the rabbit was inoculated with a virulent cultivation without being affected.

In testing the immunity it is always necessary to make control experiments, so as to be sure that the cultivations are virulent.

After inoculation with a virulent cultivation, immunity, when established, will last for a long time. I have frequently found it present 50 or 60 days after inoculation. In one of the experiments I have already quoted, it was present on the seventy-sixth day.

As a rule, when an animal has withstood inoculation with a virulent cultivation, I find it will withstand repeated inoculations, provided they are not made too quickly after one another.

THE BLOOD SERUM OF IMMUNE ANIMALS.

G. and F. Klemperer, Emmerich and Fowitzky, Foà and Carbone, were the first to show that the blood serum of immunised rabbits would protect other rabbits when injected, either at the same time or subsequently to inoculation with the pneumococcus.

Arkharow and Issaef have obtained similar results. I have made a number of experiments in this connection, injecting the serum at the same time, or shortly after inoculation, with a virulent cultivation. As a result of these experiments, I find that the blood serum of immunised rabbits sometimes protects perfectly, sometimes partially, and sometimes not at all. I have endeavoured to ascertain the conditions which give rise to the formation of a protective serum in the blood of the immunised animals.

In most of the cases, both the serum and the cultivation have been injected into the peritoneal cavity. As examples of perfect protection, I will quote the following:—

1. A rabbit weighing 1840 grms. was injected with 6 c.c. of serum and .25 c.c. virulent blood directly into the peritoneal cavity. At the same time, a control rabbit weighing 2135 grms. was injected with .25 c.c. of the same virulent blood. The control died in 31 hours of a general infection, while the protected animal was not affected, and was quite well 3 months later, having steadily gained in weight during this time.

2. A rabbit weighing 2030 grms. was injected with 10 c.c. of serum, and at the same time with .25 c.c. virulent blood, both into the peritoneal cavity. A control rabbit weighing 2525 grms. was inoculated with .25 c.c. of the same virulent blood. The control died in 13 hours of general infection. An abscess developed in the abdominal wall of the protected rabbit, but this healed, and the animal was quite well 4 months afterwards, having in the meanwhile gained in weight.

3. A rabbit weighing 2480 grms. was injected with 12 c.c. of serum, and at the same time with .5 c.c. virulent blood, into the peritoneal cavity. A control rabbit was injected with .5 c.c. of the same blood. The control died in 40 hours of general infection. The protected rabbit was alive and well 2 months afterwards.

4. A rabbit was injected with 10 c.c. protective serum and 1 c.c. virulent broth cultivation, both into the peritoneal cavity. A control rabbit of about the same weight was injected with 1 c.c. of the same virulent broth. The control died in 26 hours of a general infection, while the protected animal was alive and well a month later.

5. A rabbit weighing 2100 grms. was injected with 15 c.c. of protective serum, and at the same time with .25 c.c. of virulent blood. A control was injected with .25 c.c. of the same virulent blood. The control died in 70 hours of a general infection. The protected animal was alive and well 7 weeks later.

As examples of partial protection I will quote the following:—

1. A rabbit was injected with 10 c.c. serum and 1 c.c. virulent broth, both into the peritoneal cavity. A control was injected with 1 c.c. of the same virulent broth. The control died in 12 hours of a general infection. The protected animal appeared unaffected at first, but died 19 days later with peritonitis.

2. A rabbit was injected with 10 c.c. protective serum into the peritoneal cavity, and at the same time .5 c.c. virulent blood was injected subcutaneously. A control was injected with .5 c.c. of the same blood into the subcutaneous tissue. The control died in 18 hours, of general infection. The protected animal developed a swelling at the seat of inoculation, became emaciated, and died in 17 days. On post-mortem examination, there was a fibrinous exudation at the spot of inoculation, and peritonitis. The lymph on the peritoneum contained an abundance of pneumococci, but the heart blood only a few.

In these cases the serum only prolonged life.

I will quote the following cases to show that the blood serum of an immune rabbit sometimes possesses no protective power:—

1. A rabbit was injected with 12 c.c. serum and .25 c.c. virulent blood, into the peritoneal cavity. A control was injected with .25 c.c. of the same virulent blood. Both rabbits died in 16 hours.

2. A rabbit was injected with 10 c.c. serum into the peritoneal cavity, and with .5 c.c. of virulent blood into the subcutaneous tissue. A control received .5 c.c. of virulent blood subcutaneously. The first rabbit died in 18, and the control in 28 hours.

3. A rabbit was injected with 10 c.c. serum and with .37 c.c. of virulent blood, both into the peritoneal cavity. A control received .37 c.c. of virulent blood. Both rabbits died in 26 hours.

The protective power of the serum of immunised rabbits is of a specific character, and does not exist in the blood of normal rabbits.

I will quote experiments to show that the blood serum of a normal rabbit presents no protective properties.

1. A rabbit was injected with 10 c.c. of normal serum and .12 c.c. of virulent blood, both into the peritoneal cavity. A control rabbit received the same quantity of virulent blood but no serum. Both animals died in 15 hours of general infection.

2. A rabbit was injected with 9 c.c. of normal serum and 1 c.c. of virulent broth cultivation, both into the peritoneal cavity. A control received 1 c.c. of the same broth cultivation. The first rabbit died in 16 hours and the control a few hours later.

I thought it possible that immediately after an inoculation with a virulent cultivation the first effort of the body to resist infection would be the formation of a protective serum in the blood. The following experiment gives no support to this view:—

A rabbit was inoculated in the peritoneal cavity with a virulent cultivation. Five hours after inoculation the rabbit was bled. Twelve c.c. of the serum (which contained no cocci) was injected into the peritoneal cavity of another rabbit, together with a virulent cultivation. A control rabbit was inoculated with the same quantity of the same cultivation. Both rabbits died in 12 hours.

Now, what are the conditions which determine the protective power of the serum of immune rabbits?

In the first place, it is essential that the rabbit should be quite immune.

I believe that some of the cases where I failed to obtain a protective serum was due to the fact that the animals were not perfectly immune, although they had resisted previous inoculation.

The next point is the period at which the serum is removed after the last injection. I have generally found 8 or 9 days after the last inoculation to be the best time for removing the serum. I have not removed serum earlier than 7 days. As a rule, if serum is removed as late as 18 days it has no protective power, but I have known the serum to be protective after 19 days, and in 1 case as long as 62 days after the last inoculation.

In this latter case the test was not a very severe one, as the control rabbit did not die until 70 hours after intraperitoneal injection.

An important question arises with regard to the relationship between immunity and the protective power of the serum.

Is the former directly dependent upon the latter?

I do not feel that I am in a position to give a definite answer to this question at present.

An interesting point in this connection I have found is that the removal of protective serum from an immune animal renders the animal susceptible for a time. If it is inoculated within 2 days after removal of the serum it dies; but if the inoculation is postponed for 4 or 5 days it is not affected.

It would appear that new protective serum requires to be re-formed before immunity is again established.

THE ACTION OF THE PROTECTIVE SERUM UPON THE GROWTH OF THE PNEUMOCOCCUS.

Various statements have been made about the growth of the pneumococcus in the serum of immunised rabbits.

Some, such as G. and F. Klemperer, simply state that the serum is a good medium for the growth of the pneumococcus, and that the cultivations remain virulent. Others consider that the protective serum actually destroys the pneumococcus. Others again, such as Arkharow, Issaef and Mosny, state that the pneumococci grow in the serum in a special manner, while the virulence is not diminished.

I have made a number of experiments in this direction, and I find that the method of growth depends upon the protective power of the serum. In fact, the mode of growth in the serum appears to me to give a good indication of its protective power. If the serum presents marked protective properties the mode of growth is quite characteristic, and presents a marked contrast to the growth in normal serum.

When normal serum is inoculated with the pneumococcus it becomes quite turbid at the end of 24 hours. The turbidity is shown in microscopical examination to be due to an abundant growth of diplococci. After a few days the turbidity increases, and the serum becomes milky. When protective serum is inoculated it appears perfectly clear at the end of 24 hours, but at the bottom a sediment is seen. The sediment consists of pneumococci staining well and grouped in masses. In addition to diplococci, streptococci, often in exceedingly long chains, are seen. Sometimes only streptococci are found. If the pneumococci are transplanted to broth, an abundant growth occurs. Inoculation of rabbits with this broth shows that the virulence has not been affected.

For instance, I sowed a tube of normal serum and one of protective serum with pneumococci, and at the end of 24 hours broth tubes were inoculated from the serum tubes. After incubating for 24 hours, 2 rabbits were injected with 1 c.c. into the subcutaneous tissue. The rabbit inoculated with the culture from the normal serum died in 4 days, and the rabbit inoculated with the culture from the protective serum in 3 days. The blood of both contained many cocci.

Issaef has made extensive experiments with regard to the virulence

of cultures in protective serum, and considers that it is not at all affected.

In making these observations it is important to test the protective power of the serum. It is not enough to rely upon the fact that the animal, from which the serum is taken, is immune.

On two occasions I found that the serum of immune rabbits when inoculated became turbid; but when the serum was tested it was found not to possess protective properties. On another occasion the serum became slightly turbid, and on testing it, it was found to possess only partial protective power.

SERUM OF PATIENTS SUFFERING FROM PNEUMONIA.

Klemperer states that the serum taken from patients during convalescence from pneumonia possesses protective properties. Others deny this.

I have only made a few experiments, on account of the difficulty of obtaining serum.

In 2 or 3 cases, however, I have been able to obtain serum from blisters which have been applied during convalescence for some reason or other.

As a rule the quantity has been too small for any result to be obtained.

In 1 case 8 c.c. of fluid was obtained from a patient 17 days convalescent from pneumonia. This was injected into the peritoneal cavity, together with 0.5 c.c. of pneumonic blood. The rabbit died in 87 hours and the control in 13. The serum in this case possessed some protective power.

THE SERUM DURING THE PYREXIAL STAGE OF PNEUMONIA.

I have had no difficulty in obtaining serum from blisters applied during the acute stage of pneumonia.

In 7 cases this fluid was injected into the peritoneal cavity of rabbits, either simultaneously or some days previously to inoculation. In 1 case both animals died in 17 hours, but in all the others the animal which had received the serum died earlier, sometimes much earlier than the control.

In one case 0.5 c.c. of serum was injected into the peritoneal cavity of a rabbit 7 days previous to the subcutaneous inoculation of an attenuated cultivation; the animal died of pneumonic infection in 5 days, while the control was not in any way infected.

It would thus appear that the serum increases the virulence of the pneumococcus.

Control experiments made with blister fluid from healthy individuals showed no modification of the disease.

In concluding this paper, I feel I have added but little that is new to our knowledge. It is only because the subject has received such slight attention in this country that I have ventured to bring the results of my investigations before you to-night.¹

¹ In these investigations I have been assisted by a Government Grant from the Royal Society.

ON A SELF-ACTING MEANS FOR CULTIVATING ANÆROBIC MICROBES.

By HERBERT E. DURHAM, M.A., M.B., B.C. Cantab., *Gull Research Student in Pathology.*

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It is with great diffidence that I venture to add to the large number of "simple" methods for the culture of anærobic micro-organisms. However, since previous authors have all relied upon some special form of apparatus, my excuse shall be that anærobic cultivation may be successfully carried on with the ordinary resources of a laboratory. By the use of the following directions not only do specially and accurately ground plates, stop-cocks, etc., appear to be entirely unnecessary, but any already existing culture in tube or on plate can be treated anærobically. Those methods in which the actual culture has to be sealed up cannot but be regarded as clumsy and inconvenient, although many are the good results that they have produced. It will be best first to describe what seems to be at once the most perfect and the most simple means of obtaining a *pure hydrogen* atmosphere, and then to point out how, by slight modifications, the principle can be utilised for *impure hydrogen*, for *carbon dioxide*, or, lastly, for *pure oxygen*.

1. *Pure hydrogen*.—The means of obtaining pure hydrogen is by the action of sodium upon water, sodium amalgam (25 per cent. Na) being used on account of the too energetic action of the pure metal. The removal of oxygen is conveniently effected in a twofold manner, namely—(1) by the use of the ordinary water filter pump, and (2) by absorption with pyrogallol and caustic soda. The entrance of air is prevented by rubber bungs, and by a column of caustic soda solution. For a single tube culture the most convenient apparatus to use is formed of two large test tubes (8 in. \times 1 in.).¹

(1) A forms the *anærobic chamber*, and is fitted with a rubber cork, having a single perforation close to the side; this allows a piece of small glass tubing (the *exit tube*, *e*) to pass down to the bottom, whilst one of the ordinary culture tubes (6 \times $\frac{5}{8}$ or 6 \times $\frac{3}{4}$ in.) is contained within the large tube A. Dry pyrogallol is placed at the bottom of the large tube

¹ These are known in the trade as "boiling tubes."

A; and a small piece of iron wire gauze *d* is bent so as to form two platforms (*e.g.* like the letter "m" placed sideways), upon the lower of which a piece of sodium amalgam (about 4 or 5 grs.) is placed, while the upper supports the culture tube *g*. When charged with sodium amalgam the double platform is dropped into the tube A, as also is the inoculated culture tube. The cork, previously smeared with vaseline, is then inserted, and the exit tube *e* passing through it is so adjusted as to reach below the level of the lower platform and its contained sodium amalgam. The other large test tube B is fitted up as a "wash bottle," preferably with a rubber cork, also smeared with vaseline, the immersed tube *e'* being connected with the exit tube *e* of A, by means of a couple of inches of rubber tubing *c*. It is convenient, but not necessary, to

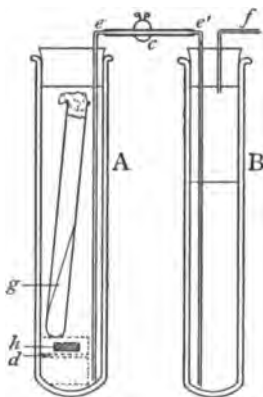


FIG. 1.

A, Anaerobic chamber. B, Reservoir. *c*, india-rubber connecting tube with clamp; *d*, double shelf of iron wire gauze; *e*, exit tube; *e'*, continuation of same; *f*, exhaust tube; *g*, culture tube; *h*, sodium amalgam.

place a screw clamp upon this india-rubber tube; a pinch-cock, or pair of Spencer Wells' forceps, or even pinching with the fingers will do, however, to occlude the passage. The tube B is filled with a solution of caustic soda, say 5 per cent., and the apparatus is ready to be set going.

There are two methods whereby the apparatus may be started—(1) by exhaustion with the filter pump; (2) by blowing down the tube *f*, and thus forcing the soda solution into the tube A. The former is my usual plan, the routine being as follows: The tube *f* is connected with the filter pump, and more or less of the contained air sucked out of the apparatus. Leakage does not

usually occur if rubber bungs are used; if present it can now be detected and remedied. The india-rubber connecting tube *e* collapses when the pressure gets low. When this has occurred the screw clamp is tightened. The tap of the filter pump is turned off, and the atmospheric pressure restored in the tube B. Then, by *cautiously* unscrewing the clamp (or relaxing finger pressure, if that method is selected), the soda solution is allowed to flow over into A. Here the soda meets the pyrogallol, and the last traces of oxygen are absorbed. When the soda solution rises a little higher the sodium amalgam is reached, and hydrogen is evolved. The clamp is again screwed up, so as to prevent soiling the culture tube with brown pyrogallol; this is not necessary, but it is advantageous, since the growth can be more readily observed, without opening the apparatus, if the tube is kept clean. When the atmospheric pressure is restored in tube A, the clamp is

loosened, and the further evolution of hydrogen causes the soda solution to flow back into the tube B. Thus the remainder of the sodium amalgam is left high, and more or less dry. This equalisation of pressure is a matter of a few minutes, and may be readily judged by the swelling of the collapsed portion of the india-rubber connection between the clamp and the exit tube *c*; or, if preferred, the clamp may be slightly loosened, and the direction of flow noted. The pressure being equalised, the two tubes A and B may be put in a rack, or fastened together with an india-rubber band, and the whole apparatus placed in an incubator.

As a refinement, the exit tube *c* may be partially drawn out in the blow-pipe flame, before the apparatus is set up; then, when no free sodium is left it may be sealed up, and the tube B removed. It is recommended that the cork of the tube A should be tied down with a piece of string in case hydrogen is still being slowly evolved. It would appear that the leakage of hydrogen through the thick rubber bung is practically negligible.

Both theoretically, and probably practically, there would be some leakage of hydrogen at the connection tube *c*; but as a matter of experience this can be avoided by adjusting the pressure so that the exit and connection tubes are filled with a column of liquid.

It need hardly be said that any two wide-mouthed bottles, one of which is capable of containing culture tubes, will answer admirably; or furthermore that, within limits, a given culture tube can be adapted by pushing in the cotton-wool plug, and cutting off the necessary length by means of a red-hot wire.

So far the premise, that no special apparatus was required, has been justified. But for plate and dish cultures, a special "jar-clamp" is suggested, since rubber bungs of more than 8 cm. in diameter are both difficult to obtain and expensive. It may be useful to others, if I give a short description of the apparatus I have devised and used. To commence with, the clamp is not an expensive apparatus, since the one I made myself cost a few pence; any blacksmith would probably make it for a shilling or two.

Two discs of sheet-iron ($5\frac{1}{2}$ in. diameter and $\frac{1}{8}$ in. thick) are each drilled with three equidistant holes close to the periphery. Through these holes are passed bolts *m*, made from $\frac{1}{4}$ -in. iron rod and about 10 in. long; the upper 4 in. is tapped with a thread, and provided with fly nuts and washers *n*. It is convenient to have a small nut *o* upon each of the bolts; by screwing these up, the upper disc is supported when the apparatus is opened. The lower end of each bolt is hammered up so as to prevent it passing through the hole in the lower disc.

A thin smearing with vaseline effectually prevents the iron from rusting.

By interposing thick rubber washers¹ above and below we have the

¹ Preferably smeared with vaseline.

means of converting wide-mouthed jars or cylinders, of very different sizes, into air-tight chambers. The cylindrical jars in which dried French plums are sold (7 in. \times 3½ in.) answer admirably; moreover, they are cheap and easily replaced if broken. They are capable of taking several 8 cm. Petri dishes, or about a dozen culture tubes.

For plate cultivations a tripod support of iron wire is stood upon the bottom of the jar; to it is fixed a bent piece of iron wire gauze to hold the sodium amalgam. The Petri dishes are piled upon a disc of tinplate, provided with three strings, or wires, so that they may be lowered down to rest upon the tripod. For tube cultures a small glass beaker, provided with a shelf of iron wire gauze, may be stood inside the

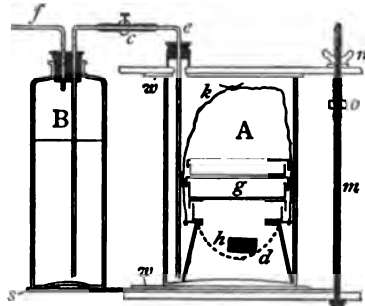


FIG. 2.

Diagram of jar-clamp apparatus in section. A, Anaerobic chamber. B, Reservoir. c, india-rubber junction and clamp; d, tripod with wire-gauze shelf; e, exit tube; f, exhaust tube; g, plate-culture dishes; h, sodium amalgam; k, strings attached to disc for removing dishes; m, one of the three bolts, with fly nut and washer n, and support nut o; s, support for reservoir clamped in under the lower washer w.

jar; the exit tube being led into this; the culture tubes may rest on the bottom without fear of soiling. The upper disc is drilled with one other hole (½ in. diameter); above and concentric to which about an inch of metal tube (1 in. diameter) is soldered. By this means the exit tube e fitted with a rubber cork can be passed down below the level of the sodium amalgam, after the jar has been charged and put into place in the clamp. It will be found convenient to place a slip of wood s beneath the lower washer w; this projects at the side corresponding to the exit tube, and forms a support for the bottle B. The whole apparatus then becomes portable, and can be moved about readily.

Simplicity of an apparatus is often inversely proportional to the length of its description; though the above may appear somewhat complex, it will be found that the apparatus is extremely simple. Experience shows that after the first charging the old dark solution may be used again and again without the addition of fresh pyrogallol. The nascent hydrogen appears to have a reducing effect upon it; so that all the time the jars are closed an oxygen absorbent is present, ready to do its work should any air enter accidentally.

When the apparatus is to be opened and the cultures examined, it is advised that the connecting tube c should be clamped; otherwise syphon action is apt to be set up, and the plugs of the culture tubes soiled when the exit tube is withdrawn.

With regard to cost, sodium is now quoted at one shilling per 30 grms. (1 oz. circ.); this will make about 120 grms. of amalgam. The mercury can be used over and over again. For the above described jar

20 grms. original charge, and additions of 5–10 grms. according to circumstances, when it is opened, will be found to suffice.

Sodium and sodium amalgam are usually supplied in naphtha; in one trial with tetanus bacillus the naphtha vapour did not appear to interfere with the growth. But I think it preferable to use vaseline as a preservative for the sodium; it prevents oxidation perfectly well.

The organism I have chiefly experimented with has been *Bacillus tetani*, which grows well in the hydrogen atmosphere thus obtained. I have not troubled to use recently boiled culture media; probably the sojourn under the low pressure of the filter pump is sufficient; bubbles are after given off from broth during the exhaustion.

2. *Impure hydrogen*.—I have found experimentally that with the apparatus arranged as above described an oxygen free atmosphere can be obtained by the action of acid upon zinc. Granulated zinc is placed in the bottom of the anærobic chamber A, and dilute hydrochloric acid in the reservoir B. The apparatus is exhausted by the filter pump, exactly as has been described above. The acid is allowed to run over, the pressures equalised, and the exhaustion repeated. Three such exhaustions and equalisations of pressure are sufficient to obtain an atmosphere so free from oxygen that a plugged trial tube (loaded with dry pyrogallol and a capsizable tube of strong soda solution) does not darken appreciably. The process does not require more than five minutes' attention. If desired, partial exhaustion may be repeated after 3 or 4 days, and a new hydrogen atmosphere established. This modification may be compared with Sternberg's device;¹ however, it has the advantage of being more portable, and is capable of being sealed up, as has already been described.

3. *Carbon dioxide*.—A slight modification will enable us to obtain an atmosphere of carbon dioxide by displacement. Instead of a single exit tube, two exit tubes are now required for the bottle A. One of these (acid exit) is arranged in the manner already described; it reaches to the bottom of the bottle A. The other (gas exit) only just passes through the cork, and is to lead off the specifically lighter gases of the contained air. Both these exit tubes are connected by india-rubber tubing with tubes reaching to the bottom of the reservoir B.

The jaw-clamp apparatus is adaptable to this modification, since a cork with two holes and tubes can be applied instead of the one with a single hole and tube. In A pieces of white marble are placed; B is filled with dilute hydrochloric acid. When the bottles are charged and corked, all that is necessary is to pinch the *gas exit* tube and blow over some acid. Carbonic acid gas is immediately evolved; the *gas exit* is released; and the oxygen, etc., of the contained air are rapidly displaced. Both zinc and marble are so cheap that it does not appear to be worth while to adopt any support to prevent the continued action of the acid. If it is desired to empty the anærobic bottle of liquid, clamping the gas

¹ Vide "Manual of Bacteriology."

exit will soon lead to this result ; by blowing over a little fresh acid the process is accelerated, if the action has become slow.

In using these methods (2 and 3) I have allowed the ends of the culture tubes to be immersed in the acid without a special support. I also cap the tubes with lead foil to guard the plugs from the spray thrown up during the effervescence.

4. *Oxygen*.—It is perhaps paradoxical to include pure oxygen in an account of anærobic methods. But the apparatus as fitted up for hydrogen can easily be adapted for oxygen by replacing the sodium amalgam by manganese dioxide, and the liquid by peroxide of hydrogen solution. I have tested the possibility of this method with glowing matches, but have not made any serious attempts to cultivate microbes under this condition.

In conclusion, I must thank Dr. Washbourn for the facilities he has given me in his laboratory, as well as for enabling me to obtain cultures of the bacilli of tetanus and malignant œdema.

In bringing forward this means of carrying on anærobic cultures, with the ordinary resources of a laboratory, I can but re-echo the hope expressed by Professor Novy, in his excellent memoir¹ that the simplification of anærobic apparatus will lead to an increase in our knowledge of these interesting organisms. I have consulted the literature of the subject, and have been unable to find record of any self-acting principle, such as has been described above.

¹ *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1893, bd. xiv. s. 581.

A MALE FŒTUS, SHOWING REPTILIAN CHARACTERS IN THE SEXUAL DUCTS.¹

By SAMUEL G. SHATTOCK, F.R.C.S.

(PLATE XVI.)

BEFORE describing the condition of the sexual ducts, it may be remarked that the abnormalities in question are associated with ectopia of the urinary bladder and epispadias, and that for this reason the ducts themselves like the ureters open on a free surface.

The ectopia of the bladder is associated, moreover, with prolapse of the intestine, which has taken place immediately above the extroverted bladder. There is thus a continuous free mucous surface comprising the bladder and everted intestine.

Below and behind the convexity of the general protrusion is an empty scrotum, and the rudiment of a penis, as in an ordinary case of epispadias. There is no intestine within the pelvis, and no anus.

Kidneys.—The kidney of the left side is normal; that of the right side so remarkably elongated that its lower end reaches far into the cavity of the pelvis. The contrast will appear from the following measurements: the left from upper to lower extremity is 3·5 cm. in length; the right, 5·2 cm.; there is, nevertheless, no real excess in its volume.

The elongated kidney (which, like the left, is remarkably lobulated) has a single ureter, but the upper end of this is subdivided into three main branches, in correspondence with the abnormal figure of the organ. Each ureter opens on the free extroverted surface of the bladder at the upper angles of an area corresponding to the trigone.

Testicles.—On each side within the lower part of the abdomen there is a well-formed testicle with epididymis and vas deferens.

The vas of the left side opens into the ureter about 1·4 cm. above the external orifice of the latter. On the right side the lower end of the vas is traceable by dissection slightly beyond the corresponding ureter, lying to its inner side without opening into it; its termination becomes lost in the tissues beneath the peritoneum.

The *Müllerian ducts*, the persistence of which constitutes another of

¹ Communicated to the Pathological Society of London, 19th February 1895, and published with permission of the Council.

the valuable facts in the malformation, lie longitudinally, one on either side of the middle line. The upper end of each is bent sharply forwards and downwards upon itself for about 1.5 cm., and terminates in a blind rounded extremity. The upper end of the right is contiguous with the lower extremity of the kidney; between the upper end of the left and the corresponding kidney there intervenes a distance of 1.1 cm.

Each tube has an average diameter of 6 mm., except at the highest procurved part, where it is somewhat narrower, and from its upper point of duplicature to its lower opening the length of each is about 3 cm.

Inferiorly the ducts open on the exterior. Their openings lie to the inner side of and slightly behind the ureters; between the apertures of the persistent ducts themselves there is an interval of 1 cm.

A thick bundle of nerves extends from the hypogastric plexus to the tubes under consideration, the distribution of the nerves being chiefly to the upper part of each. Microscopic examination proves the fibres to be of the non-medullated kind.

A short way in front of the openings of the persistent Müllerian ducts are two others of lesser diameter, one on either side, which lead each into a narrow tube quite distinct from the first named. These lesser tubes lie longitudinally against the Müllerian ducts, are 1.7 cm. in length, and terminate in free blind upper extremities.

Remarks.—The prolapse of the intestine above the extroverted bladder obviously concerns the blind termination of an imperforate large intestine. The prolapse is not more than 3.2 cm. in length, but this represents all that is developed of the gut on the distal side of the umbilicus, i.e. of the omphalo-mesenteric duct. There is an aperture on the upper aspect of the root of the prolapsus which leads into the general small intestine.

In the forty-second volume of the Pathological Society's *Transactions*, Mr. William Anderson has reported the case of an infant (the specimen of which is in St. Thomas's Hospital Museum), in whom an umbilical fistula was associated with a similarly ill-developed condition of the large intestine; the latter terminated in a free blind extremity within the abdominal cavity, and did not exceed 18 cm. in length. This prolapse of the bowel, in association with extroversion of the bladder, is a well-recognised teratological condition. I have seen it in another instance (lately added to the Hospital Museum), where the eversion concerns chiefly the upper segment of intestine above the umbilicus, and is associated with an imperforate condition, but only slight prolapse, of the lower; both segments open freely about the upper part of the extroverted bladder. In this specimen the upper prolapse has an extent of 4 cm.; the blind distal segment, of 10 cm., and within its umbilical opening lies the orifice of the appendix vermiformis; the exposed mucosa of the bladder is much thickened, and has in some parts a close-set papillary surface, whilst in others, though equally thick,

it is smooth, as in a case of extroversion I have described in the forty-fifth volume of the Pathological Society's *Transactions*; the increase in the first instance is brought about by up-growth on the papillary type, in the second by down-growth on the glandular. The double prolapse has obviously taken place through the omphalo-meseraic duct, which seems to have been involved in the fissure affecting the urinary bladder.

Forster¹ gives figures, collected from various sources, of a similar condition; in some the upper segment is prolapsed, in others it is not. He classifies them all as examples of cloaca-formation, associated with fissure of the bladder. This, however correct in a physiological sense, is misleading and erroneous in the more important morphological one.

Anatomically, there is nothing homologous with a cloaca in this complication of ectopia; the large intestine is *imperforate* at its *distal* extremity; it opens, so to speak, at the wrong end, not into a cloaca, or on a cloacal surface, but about the umbilicus in the region of the omphalo-meseraic duct.

Ahlfeld² gives similar figures. Nevertheless, what is morphologically a true cloaca may be present in cases of ectopia vesicæ, even where there is no proper anus,—if, for example, the normal developmental communication which exists between the front of the hind gut and the uro-genital sinus persist. Such a persistence is quite common in cases of imperforate rectum in the male (recto-urethral fistulæ), and occurring in conjunction with ectopia vesicæ would constitute a true cloaca.

The morphological test, in short, turns upon which end of the large intestine is open; if the distal end is absolutely closed there cannot be a cloaca, if this end opens on the mucous surface of the fissured uro-genital space or bladder, there is.

The remarkable elongation of the right kidney recalls the reptilian condition, such as is met with especially in the crocodile, in Ophidia and many Lacertilia; and it is of interest in conjunction with the corresponding reptilian disposition of the different sexual ducts to be immediately noticed. What is particularly remarkable, too, is that the elongation affects the *right* kidney, for in Ophidia the right kidney is much longer than the left. The elongated form of the kidney in reptiles is apparently related to that of the trunk; it is most pronounced in Ophidia, less so in Crocodilia and Lacertilia, whilst in Chelonia the organ is almost discoidal. So amongst Amphibia, the kidneys of the frog are not strikingly long; in the newt they measure about a fourth of the entire body length, excluding the tail.

But to come to the ducts described one on either side of the middle line. These cannot be regarded as other than persistent Müllerian ducts, which have retained, throughout, their primitive distinctness; there is nowhere any coalescence to form a uterus or vagina.

¹ "Missbildungen des Menschen," Tafel xxii.

² "Die Missbildungen des Menschen."

As I have elsewhere remarked,¹ it is a noteworthy circumstance that in cases of such persistence in the male, whether on one or both sides, the upper end of the duct is not found related to the testicle, but to the kidney, although seeing that the hydatid of Morgagni is held to represent the upper end of the Müllerian duct it would be natural to suppose that when persistent the duct would retain its relationship with the sexual gland during the descent of the latter into the scrotum.

In the present instance, the duct of the right side is contiguous with the lower end of the kidney, but on neither side is it related to the epididymis or body of the testicle. Is there an hydatid on the testicle?

It is of paramount importance in this connection to distinguish true and false hydatids.

Let it be assumed, then, that *the* hydatid projects from between the globus major of the epididymis and the body of the testicle, and that such as project from the body of the testicle itself, or from the upper surface of the globus major represent free ends of the Wolffian tubuli, what does the present specimen show?

In the paper last referred to I have pointed out that in one case, the hydatid is stated to have been absent; in another it is recorded as having been present on the side corresponding with the persistent Müllerian duct, and absent on the opposite,—there is a strong temptation to assume an erroneous transposition of "right" and "left" in this report, but so it stands, and so the specimen is figured.²

I may here state the results of a careful examination of a series (12) of foetal testicles at different ages, which I made in order to observe the disposition of the hydatids. In one, and only one, was there a second hydatid; in this case *the* hydatid was a well-formed leaf-like appendage attached to the anterior edge of the globus major; the second, quite minute and spherical, was attached to the globus just above its anterior edge and to the inner side. In the other specimens the attachment of the hydatid lay in the fissure between the globus and the summit of the body of the testicle; sometimes it was to the under aspect of the globus, but usually to the bottom of the fissure; in the smallest specimen the body of the testicle measured 5 mm. in length, the hydatid (visible only through a lens) was fixed as last mentioned.

So far now as concerns the malformation under consideration, I may describe precisely the condition met with. On both sides there are hydatids. On the right side there is one hydatid attached to the summit of the body of the testicle; a second, to the upper surface of the globus major, a short way from its extreme anterior margin; a third, more minute, to the line of junction between the body of the testicle and the globus major, there being no fissure between these parts as there usually is in the foetus.

On the left side there are two minute hydatids attached to the

¹ "Parepididymal Cyst," *Trans. Path. Soc. London*, vol. xlii.

² Case by Chas. Rémy, *Journ. de l'anat. et physiol. etc.*, Paris, 1879.

anterior border of the globus major at the site of its adhesion with the body of the testicle, there being an absence of the usual fissure on this side as on the other. Whether *the* hydatid can be regarded as present, I do not pretend to say; the result of the explanation is ambiguous and unsatisfying.

Had there been no extroversion of the bladder, the persistent ducts would have opened into the uro-genital sinus, for their orifices lie slightly below, or, were the extroverted parts reduced, in front of, the ureters.

The complete independence and persistence of both ducts throughout their course must be regarded as a reversion to the reptilian type, where both oviducts open into a cloaca.

A similar persistence, of course, obtains in the Monotremata, Ornithorhynchus, and Echidna, but these characters are so essentially reptilian that they really relate the Monotremata to Reptilia; and the malformation under consideration is more correctly relegated to the last than to the lowest mammalian form. The justness of this will appear, also, in the disposition of the vas deferens to be presently noticed, which is one not found in Monotremata but confined to Lacertilia.

As to the blind smaller tubes by the sides of the Müllerian ducts, these, it must be inferred by analogy, represent the structures so constant in certain groups of Reptilia, namely, the anal pouches which open into the cloaca.

The right pouch in the fœtus under consideration I examined by microscopic section. The wall presents a well-marked circular and longitudinal layer of unstriated muscle fibre, and to the inner side of this a mucosa of lymphoid tissue in which lie somewhat closely set, simple tubular glands or crypts, lined with a single layer of perfectly developed columnar epithelium.

There is finally another persistent embryonic feature, still more interesting, and again explicable on the principle of atavism, or reversion to an ancestral type.

In the human subject the ureter is normally developed as a diverticulum from the Wolffian duct. On the left side this primitive relationship persists; the vas deferens opens on the surface in common with the ureter, or, to speak more accurately, the ureter stills opens into the Wolffian duct, the original continuity of the two canals not having been lost by any subsequent dislocation in the course of development.

The arrest at this stage may be explained, then, as arising in a reversion to an antecedent reptilian type; in the same way that one so explains many of the anomalies affecting the cardiac cavities. Even what is usually regarded as a strictly pathological condition, namely, the

extreme forms of hypospadias, may, it seems to me, be viewed in the same light; for among reptiles, the penis of *Chelonia* and *Crocodylia*, which is single and not paired as in *Ophidia* and *Lacertilia*, is not perforate but merely grooved on the posterior or lower aspect and attached to the anterior wall of the cloaca.

This commonness of the ureter and vas deferens is distinctly a

reptilian condition. In the male of *Monotremata*, the renal and seminal ducts open separately into the cloaca, as they do also in birds and most reptiles. But among *Reptilia*, lizards present precisely the condition shown in the specimen, *i.e.* the ureter and vas combine near their lower terminations and open through a single aperture into the cloaca. I have ventured to figure this disposition from a particularly well-marked instance.

Although the rule in *Lacertilia*, it is not, however, absolutely constant; Owen¹ describes the openings as distinct in *Lacerta ocellata*.

In the male of *Amphibia* the vasa efferentia are in communication with the kidney, through which the seminal products make their way, an arrangement considerably lower than that under consideration.

As to the unusual opening of the rectum above or in front of the apertures of the Müllerian or oviducts, such a position is met with in certain reptiles (*Ophidia*, *Chelonia*), but no true homology underlies the condition in the present case.

The intestinal aperture, as pointed out in the earlier part of this paper, does not correspond with such as would communicate with a cloaca; the distal end of the large intestine is blind, and the external opening concerns not this but the proximal



FIG. 1.—The genito-urinary organs of *Varanus Salvator*. The parts are viewed from the dorsal aspect. On either side are shown a testicle with the vas deferens, and below the testicle the much subdivided kidney. Below the kidney is the straight continuation of the vas, and, to its outer side, the ureter; these unite into a common duct before opening into the cloaca (Mus., Royal Coll. Surg., London).

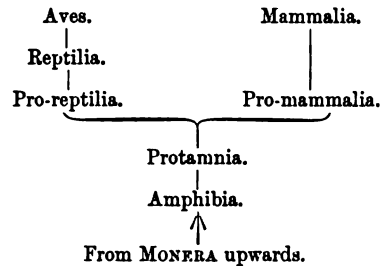
one, the bowel being prolapsed at the umbilicus, in the neighbourhood of the omphalo-meseraic duct.

I have throughout referred the malformations in question to the reptilian type, because they are most conformed to this.

¹ "Anatomy of Vertebrates," pp. 5 and 6.

Hæckel,¹ in tracing the mammalian pedigree, after starting from the lowest invertebrate forms, at length reaches the Amphibia. From these he makes two diverging lines of descent, to the one side Mammalia, to the other, Reptilia, and, beyond Reptilia, Aves. Not a few missing links there are in the pedigree, provisionally filled in. Thus above the Amphibia he places next in succession a hypothetical series of Protamnia, in which are included the primary forms of the three higher classes of Vertebrata, namely, mammals, reptiles, birds; and yet two further hypothetical groups before the proper Reptilia and Mammalia, Pro-reptilia and Pro-mammalia. The Protamnia, Hæckel considers to have been most closely related to the existing Ornithorhynchus and Echidna.

Mammalian Pedigree, constructed after Hæckel.



Assuming this scheme to be correct, all I can suggest is, that if the reversions in the present instance are not strictly reptilian, they are related to some of these missing groups; the reptilioid characters which they disclose might have pertained to the common ancestors of modern mammals and modern reptiles; they are lower than those of the lowest existing mammals (Monotremata), yet higher than those of existing Amphibia.

At the present time, however, there is a general agreement to relate Reptilia and Mammalia more closely, if not to regard the mammalian stem as terminating at Reptilia rather than Amphibia.

One of the more remarkable recent discoveries is the well-known fact that the ova of Ornithorhynchus are provided with a shell, and hatched, like those of reptiles, outside the body. Professor H. G. Seeley, in a paper on the "Nature and Limits of Reptilian Characters in Mammalian Teeth,"² remarks that if the tooth of the Ornithorhynchus cannot be exactly paralleled in any other animal, it is at least evident that the teeth are as reptilian as the skeleton; that there are several features in which the teeth of reptiles and mammals resemble each other morphologically; and that the lower mammals emphatically approach towards reptiles in all essential characters of tooth form. Of teeth he gives six

¹ "History of Creation," 1876.

² *Proc. Roy. Soc. London*, 1888, vol. xlv.

typical characters which are regarded as mammalian,—the presence of more than one root, etc., and proceeds with the statement that no one of these is constant in the class, and its loss is in every case an approach towards a reptilian type.

The same authority has, moreover, drawn attention to the skeletal affinities between reptiles and mammals, and after noticing that the most ancient mammals exhibit resemblances to monotremes, edentates, insectivores, and apparently carnivores, observes that it is among these orders that the closest correspondence is found, bone for bone, with reptiles. Seeley, nevertheless, is driven by the force of facts very close to Hæckel's scheme, as the following quotation will show:—"The oldest known fossil representatives of both groups (*i.e.* reptiles and mammals) certainly approximate closer towards each other in all known parts of the skeleton than do the orders which survive; so that it may be a legitimate induction that, in an earlier period of geological time, the characters of both groups were so blended that there existed neither the modern reptile, which has specialised by losing mammalian attributes, nor the modern mammal, which has specialised by losing the skeletal characters which have come to be regarded as reptilian." In short, Hæckel's *Protamnia* are reptilian in type.

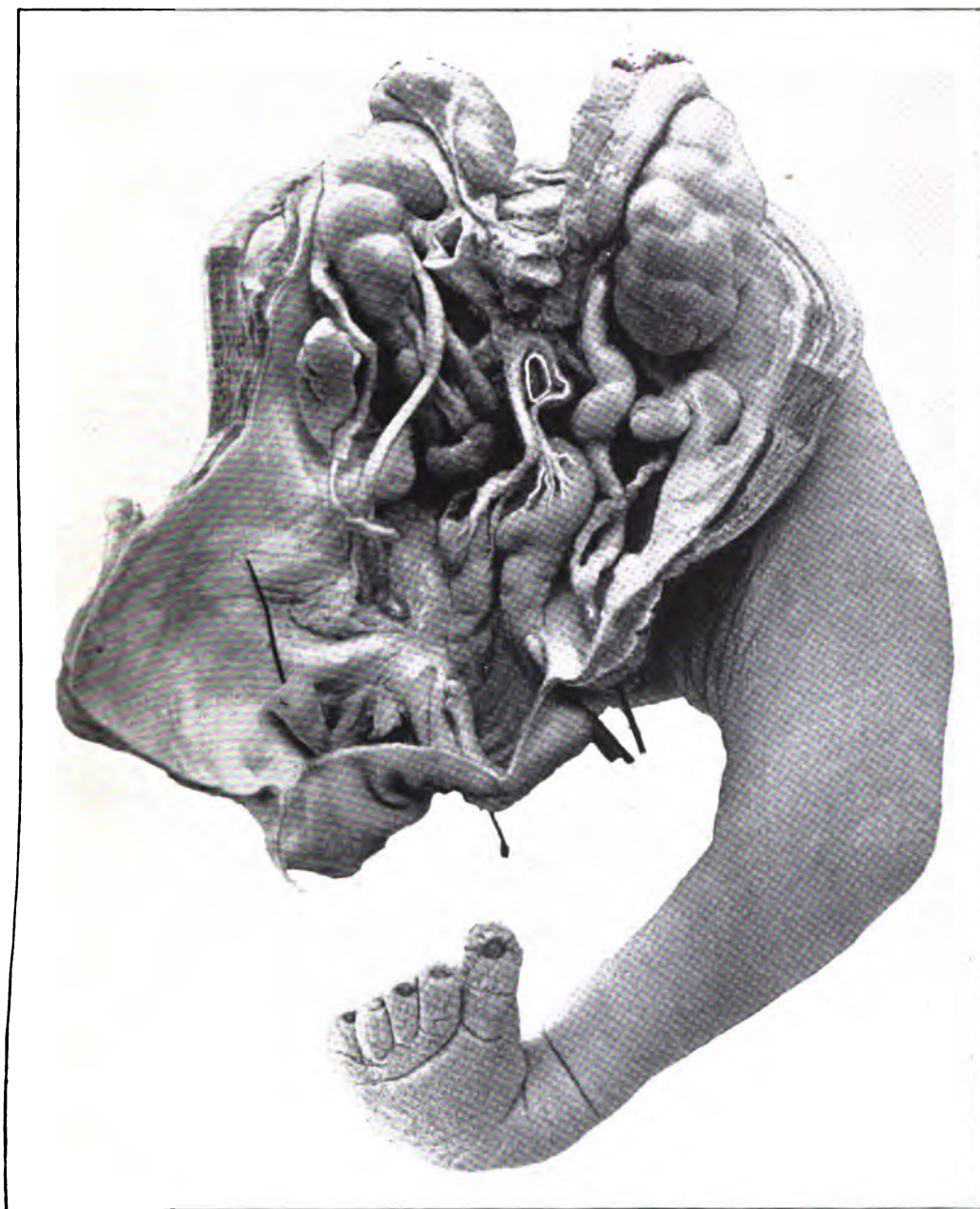
And this view of the mammalian pedigree is borne out by the remarkable facts disclosed in the malformation described, which in a certain sense confirm the conclusions not only of more recent zoology but those of palæontology.

DESCRIPTION OF PLATE XVI.

The malformations described are complicated with ectopia of the bladder and imperforate rectum, the latter being everted and prolapsed at the umbilicus. There are shown—

1. The elongation of the right kidney.
2. The persistence of both Müllerian ducts, which remain distinct throughout their course, and open near the ureters on the exterior of the extroverted parts.
3. The union of the ureter and vas deferens (on the left side), such as is met with in *Lacertilia*.
4. The presence of two independent sacs, representing anal pouches; these lie in front of the lower ends of the Müllerian ducts.

The parts are shown of the natural size.



ABSORPTION AND METABOLISM IN OBSTRUCTION OF THE PANCREATIC DUCT.

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Grocer Research Scholar.*

CLINICAL observers and experimental pathologists have, during the last few years, thrown much light on the true pathology of diseases of the pancreas; so that we are now in a position to diagnose cases which formerly would only be recognised by post-mortem examination.

We know that in all cases where, either from disease or the artificial removal of the pancreas, it is entirely excluded from the organism, sugar invariably appears in the urine (*vide* von Mering and Minkowski,¹ Lépouin,² Hédou,³ Vaughan Harley,⁴ etc). In cases of total extirpation of the pancreas, sugar only disappears from the urine when the animal suffers from some inflammatory disease (pneumonia, peritonitis, etc.), or coma is about to make its appearance, as indicated by an increased excretion of ammonia in the urine, as well as acetone, aceto-acetic acid, or even by oxybutyric acid.

On the other hand, in experimental cases, where even only a small portion of the pancreas is left, we invariably get no sugar in the urine, or, at the most, a transient glycosuria caused by the operation. In those cases only when the animals are rapidly losing flesh does acetone or aceto-acetic acid appear in the urine.

In the human subject, when the pancreatic duct from any cause (inflammatory obstruction or concretion, or pressure from malignant disease, etc.) is obstructed, and only the pancreatic juice prevented from reaching the intestines without destruction of the gland itself, no sugar appears in the urine. These cases correspond to the animals in which the gland is only partly removed, and are extremely difficult to diagnose.

Consequently, it is of particular interest to investigate what changes occur in the absorption of food, as well as in metabolism in that dis-

¹ Von Mering and Minkowski, *Arch. f. exper. Path. u. Pharmacol.*, Leipzig, 1889, bd. xxvi.

² Lépouin, *Compt. rend. Soc. de biol.*, Paris, Avril 1890.

³ Hédou, *ibid.*, Oct. 1890.

⁴ Vaughan Harley, *Journ. Anat. and Physiol.*, London, 1891, vol. xxvi. p. 1.

eased condition in which so important a juice as that of the pancreas is prevented from entering the alimentary canal.

Since as early as 1832, Bright¹ stated that, in diseases of the pancreas, large quantities of fat were found in the stools. In 1862, George Harley² pointed out that the solidification of oil taken by the mouth in the stools was a reliable sign of occlusion to the pancreatic duct. Experimentally, von Mering and Minkowski³ showed that in dogs in which the pancreas had been entirely extirpated, and the animals thus rendered diabetic, large quantities of fat occurred in the stools.

Subsequently, Abelman⁴ investigated the metabolism of dogs from which the pancreas was either partially or totally extirpated. In this research he showed that von Mering's and Minkowski's observations were perfectly correct, and that animals thus operated on were unable to absorb as much fat as normal animals. In fact, olive oil was entirely unabsorbed, and milk fat was only absorbed in small quantities. He further found that not only was the absorption of proteids very much diminished, but carbohydrates were not absorbed in the normal quantity.

In this paper it is my intention to describe the results of an investigation into the absorption of food in a human subject in which the pancreatic duct was probably obstructed. I shall first give a résumé of the results I have obtained in the case of dogs, in which the pancreas has been either partially or completely extirpated, in order to show that the results obtained in the human subject correspond to those known to occur in experimental cases.

In all the cases in which the pancreas had been completely removed, as has been already stated, sugar occurred in greater or less quantity in the urine; even if a dog was kept fasting 5 days, the urine contained from 1 to 7 per cent. of sugar. Polyuria, although never very marked, now and again showed itself. The quantity of nitrogen in the urine was always in excess of the quantity really absorbed, and was accounted for by the rapid emaciation. Dogs in which the pancreas had been removed lost from $1\frac{1}{2}$ to $3\frac{1}{2}$ kilos. during 4 days' fasting; while only in one case did a dog in which no operation was performed lose 1 kilo. during the same time. In most cases both acetone and aceto-acetic acid appeared in the urine before death.

If the pancreas was only partly removed, there was no glycosuria; or, at the most, only a transient one, immediately after the operation. The quantity of nitrogen in the urine was not so markedly increased, and the emaciation more gradual, large quantities of food sometimes being able to maintain the body weight for some time. Acetone and aceto-acetic acid appeared only in the urine when the animals had

¹ Bright, *Trans. Med.-Chir. Soc. Edin.*, 1832.

² George Harley, "Complete Obstruction to the Bile and Pancreatic Ducts," *Trans. Path. Soc. London*, 1862, vol. xiii, p. 118.

³ Von Mering and Minkowski, *Arch. f. exper. Path. u. Pharmacol.*, Leipzig, 1889, bd. xxvi. s. 371.

⁴ Abelman, "Inaug. Diss.," Dorpat, 1890.

considerably lost flesh. The fæces of all the animals that I have experimented upon have had a most peculiar smell; this fact, trifling as it may appear, is nevertheless of interest, as I have found it invariably to occur in animals, and it has also been present in the case about to be described.

When the dogs were fed on raw meat, this very often reappeared in the stools apparently unaltered; when fat was given, this also appeared in large quantities in the stools.

Before describing the results it will be as well to give in a few words the methods employed in analysis in the case of the dogs and boy.

With the first meal a quantity of charcoal was given, so that the fæces of that meal might, by their dark colour, be distinguished from those belonging to the previous diet. With the last meal charcoal was also given for the same purpose. In the case of the dogs, they were kept fasting before commencing diet, in addition to their getting charcoal. The quantity of nitrogen was always estimated by the method of Kjeldahl. In the case of the food and the fæces, at least four samples were analysed, and the quantity of nitrogen calculated from the average. In the urine two analyses were found sufficient.

In analysing the fats, some of the fæces were first extracted with alcohol and then with ether in a Soxhlet's extractor; and the two extracts, after being dried, were again treated with absolute ether, and then the extract was weighed. The residue, after complete extraction with ether, was heated with dilute hydrochloric acid, dried, and again extracted with ether, so as to obtain the fat acids liberated from the soaps. The quantity found in the first ether extract, together with the soap, is termed total fat in the following table.

In order to separate the neutral fat, fat acids, and cholesterin, the first ether extract was warmed with a solution of sodium carbonate to saponify the free fat acids; and, after drying, the neutral fat and cholesterin were extracted with absolute ether. The free fat acids were calculated by the loss of weight.

The new ether extract was then treated with a freshly-prepared alcoholic solution of metallic sodium (NaOH), and, after drying, extracted with ether to separate the cholesterin. It was found in practice often necessary to repeat the process several times before pure cholesterin could be obtained, and in all cases it was repeated until there was no longer any loss of weight. By subtracting the quantity of cholesterin from the neutral fat and cholesterin, the quantity of neutral fat was obtained.

In order to study the absorption of milk fat and proteids in the case of the boy, a quantity of milk was sterilised, and a litre analysed to form a standard of comparison. The method of analysis was exactly similar to that employed in the case of the dogs.

The results of the analysis on one dog, which lived for 2 months after an almost total extirpation of the pancreas, will now be given as

an example. The dog had lost flesh considerably, although the urine at no time contained any sugar, for which it was repeatedly examined by fermentation, phenylhydrazine, and Fehling's tests. The urine, on the other hand, contained not only acetone but aceto-acetic acid in small quantities. The fæces always contained undigested food, and had the remarkably foul odour above described.

TABLE I.—*Absorption of Nitrogen in a dog after Extirpation of the Pancreas, the Animal having considerably lost flesh, but the Urine never containing Sugar, due to the fact of very small portions of the tail of the Pancreas having been left.*

Day.	Weight.	Food.	Urine.	Fæces.	Absorbed.		
		Nitrogen.	Nitrogen.	Quantity.	Nitrogen.	Total Nitrogen.	Nitrogen per cent.
1	Kilos. 5·700	Grms. No food	Grms. 1·945	Grms. ...	Grms. ...	Grms. ...	Grms. ...
2	5·700	} 17·618	2·198	80·2	5·900	} 14·469	} 17·88
3	5·500		2·260	60·3	3·100		
4	5·450		3·013	66·4	2·221		
5	5·300	No food	1·147	67·0	3·248	}	}
6	5·150	Do.	1·520	18·7	0·644

On the first day (Table I.) no food was given, while during the next 3 days the animal was given meat containing 17·618 grms. of nitrogen. With the first and last meal charcoal was given to indicate how long the fæces passed belonged to this diet, the black colour disappeared on to the sixth day. The stools passed on the sixth day, as they did not contain any charcoal, are not reckoned in calculating the total quantity of nitrogen unabsorbed.

That fasting animals pass fæces, we know from the experiments of Voit, and that the same holds good in human beings is still further shown by observations made on various fasting individuals. The fæces passed on the sixth day correspond in all probability to the fasting stool, which is supposed to be derived from an excretion into the alimentary tract, as described by Hermann, Ehrenthal, Bernstein, and F. Voit. It is interesting to note that when the food was given on the second morning, the animal passed some of the black-coloured stools in the evening. The stools were each day collected in the morning before the food was given, but for the purpose of convenience they are entered in the above table as if passed during the same day.

It is seen in Table I. that the quantity of nitrogen given in the meat was 17·618 grms., while the quantity eliminated from the alimentary canal was 14·469, consequently only 17·80 per cent. of the nitrogen

given was absorbed. In reality, in all probability, somewhat more was absorbed, as some of this nitrogen found in the fæces would correspond to that which is normally eliminated in a fasting animal.

In the analysis of the urine, we find that during the 3 days on which the animal was fed the quantity of nitrogen eliminated was almost uniform; at the same time the animal could not be said to be on nitrogen equilibrium, as it continuously lost weight.

The quantity of nitrogen eliminated in the urine and fæces was more than was really given to the animal in the diet, so that some of the nitrogen contained in the urine was derived from a breaking down of the tissues themselves, hence the loss of weight.

The loss of weight in this experiment may be partly explained by the diminished absorption of food from the alimentary canal, but this alone does not seem a sufficient explanation.

Abelmann found that when he removed the entire pancreas, 22 to 58 per cent. of the proteids given to his dogs were absorbed, while, when the gland was only partially removed, the absorption ratio rose from 40 to 83 per cent.

It is therefore brought out by these experiments that the absorption

TABLE II.—*The Absorption of Mutton Fat after almost complete Extirpation of the Pancreas, one-twentieth of the Tail of the Pancreas being left.*

Day.	Weight.	Fat in Food.		Fæces.		Absorbed.	
				Quantity.	Fat.	Fat.	Fat.
	Kilos.	Grms.	Total Grms.	Grms.	Grms.	Total.	Per Cent.
1	7.670	12.08	36.40	19.3	2.25	26.71	26.62
2	7.600	24.32		106.0	24.461		
9	6.850		75.81	116		47.05	37.94
10	6.830			174			
11	6.720			53			
15	6.500		46.95	None.		44.885	4.44
16	6.350			9.620			
17	6.250			8.037			
18	6.100			9.914			
19	5.900			10.936			
20	5.900	No food.		6.378			

of proteids from the alimentary canal are markedly affected in pancreatic disease, and that, as will be seen later, they are in reality not much less affected than the absorption of fat which has hitherto been generally believed to be, if not the only, at least the principal one affected.

If we now turn to the effects on the absorption of mutton fat, produced by extirpation of the pancreas in dogs, we find that the proportion varies with the period which has elapsed since the operation.

Charcoal was given as indicator in this case, in the same manner as previously described. In the above table it is seen that in the first two periods of 2 and 3 days respectively, 26·62 to 37·94 per cent. of the fat given was absorbed; the largest absorption taking place when the largest quantity of fat was given. While during the third period, when the animal was in a weaker condition, and would not take so much food, the quantity of fat absorbed was much less, being only 4·44 per cent.

Abelmann found that after the total extirpation of the pancreas in his dogs, no fat was absorbed; whereas, in partial extirpation, from 25 to 59 per cent. of the quantity given was absorbed. Milk fat, however, proved an exception to this rule, for he found that in total extirpation 28 to 53 per cent. of milk fat was absorbed.

In other experiments, in which I have endeavoured to ascertain the amount of fat absorbed from the intestinal canal of dogs, after either partial or total extirpation of the pancreas, I have in all cases found a very marked decrease from the normal amount of fat absorbed, but the above samples are the best of them.

In a paper on the absorption of milk fat, recently published in the *Journal of Physiology*,¹ I showed that while a normal dog fed on milk absorbs 21 to 46 per cent. of the fat given in seven hours, in a dog from which the pancreas had been entirely removed no absorption of milk fat whatsoever could be found to have occurred in this space of time.

With these preliminary remarks, I will proceed to narrate an exceptionally characteristic case of pancreatic duct obstruction in which, through the kindness of Dr. Auld of Wimborne, I had the opportunity of making a series of analyses while on a fixed diet.

The patient was a boy aged 13, who was attacked by severe gastritis, after recovering from scarlet fever, in February 1894. He had previously suffered from an attack of acute nephritis. Two months after the scarlet fever, an offensive smell was noticed by persons coming near him, and this was found to be due to an oily excretion which collected on the seat of his trousers.

On examining the *fæces*, Dr. Auld found them of a light brown colour, soft, and containing undigested food. A large quantity of oil floated about them; on cooling, the oil solidified into a hard beeswax, like cake. A motion followed immediately upon each meal, associated

¹ Vaughan Harley, *Journ. Physiol.*, London, 1895, vol. xviii. p. 1.

with pain in the rectum, which was found to be due to inflammatory congestion of the mucous membrane and an appearance of villous growths.

When Dr. Auld brought the boy to Dr. George Harley on the 4th October 1894, he was passing, every tenth day or so, a large quantity of more or less bright orange-coloured oily fluid, which immediately gave rise to the supposition that he was labouring under some form or another of pancreatic disease. His abdomen was consequently carefully examined, without any pain, tenderness, or swelling of any kind being found in the pancreatic region. The oily stools were, however, so characteristic of the absence of pancreatic juice that he was put under the appropriate treatment for that affection.

When I examined the urine in June it contained 1.51 per cent. of urea, no acetone or aceto-acetic acid, nor any sugar. At this time he weighed 78 lbs. Some of the oily matter he passed was sent to me for analysis in July, and I found that it consisted of small quantities of neutral fat and soap, and large quantities of fat acids.

During December 1894 all medicines were stopped, and the patient was placed for 4 days on an entirely milk diet.

On this diet the fæces were of a yellowish-white colour, and of the consistence of a soft cream cheese. They contained a few yellowish lumps like beeswax, and smelt like extremely bad cheese. They contained a small quantity of bile acids and urobilin. From this, and the absence of any jaundice or bile in the urine, the bile duct was evidently pervious. The results of a quantitative analysis are given below in a tabular form.

TABLE III.—*Boy, æt. 13, suffering from probable Obstruction to the Pancreatic Duct.*

Date.	Weight.		Milk Diet.				Urine.				Fæces.			
			Quan- tity.	Nitro- gen.	Pro- teids.	Fat.	Quan- tity.	Reac- tion.	Sp.gr.	Nitro- gen.	Quan- tity.	Nitro- gen.	Pro- teids.	Fat.
Dec. 13,	Kiloes 37.8	Lbs. 84	C.c. Ordinary	Grms. 13.12	Grms. 82.5	Grms. 196.85	C.c.	Grms. 10.047	Grms. ..	Grms. ..	Grms. ..	Grms. ..
„ 14,	37.8	84	3976	13.12	82.5	196.85	1680	Acid.	1007	10.047
„ 15,	37.6	83.5	3976	13.12	82.5	196.85	1960	Do.	1007	10.272	257.84	1.149	8.931	19.98
„ 16,	37.4	83	3976	13.12	82.5	196.85	2240	Do.	1008	12.096	688.02	5.681	35.104	149.72
„ 17,	37.4	83	3976	13.12	82.5	196.85	1960	Do.	1009	10.027	576.40	4.859	30.368	137.87
„ 18,	37.6	83.5	Ordinary	diet.
„ 19,	37.8	84.0	Ordinary	diet.

The analysis of the 4 days given in Table III., shows that as far as the urine is concerned, the quantity of nitrogen excreted is fairly equal. During the first 2 days, on a milk diet, he lost a pound in weight (14th to 16th), the next 2 days (from the 16th to 17th) his weight remained the same, and the nitrogen eliminated in the fæces and urine was prac-

tically equal. In calculating the absorption and metabolism in his case, we can only employ the results of the 16th and 17th of December.

As regards the absorption of nitrogen and fat from the alimentary canal, it will be as well to compare the average of these 2 days with that found by Rübner¹ in a healthy man on a milk diet.

TABLE IV.—*Comparing the quantity of Nitrogen and Fat absorbed from the Alimentary Canal on Milk Diet in a healthy Man (Rübner), and one suffering from probable Obstruction to the Pancreatic Duct.*

Condition.	Milk contained.			Fæces contained.				Absorbed.			
	Quan- tity.	Nitro- gen.	Fat.	Nitrogen.		Fat.		Nitrogen.		Fat.	
	C.c.	Grms.	Grms.	Grms. Total.	P.Ct.	Grms. Total.	P.Ct.	Grms. Total.	P.Ct.	Grms. Total.	P.Ct.
Health, . . .	3075	19·4	119·9	1·5	7·7	6·7	5·6	17·9	92·3	112·9	94·4
Pancreaticobstruc- tion, . . .	3976	13·12	196·85	5·25	40·0	143·80	73·05	7·87	60·0	52·05	26·95

In Table IV. it is seen that in a healthy man the fæces contain 1·5 grms. of nitrogen, *i.e.*, 7·7 per cent. of the total nitrogen given, consequently 92·3 per cent. of the total nitrogen given has been absorbed. When we compare this with the case under investigation it is seen that in an average of 2 days, during which the patient was on nitrogen equilibrium, the fæces contained 5·25 grms. of nitrogen, so that 40 per cent. of the total nitrogen given was eliminated in the fæces, and only 60 per cent. had been taken into the system to be made use of in metabolism.

As regards the fat, in Rübner's healthy man only 6·7 grms. were excreted in the fæces, *i.e.*, 5·6 per cent.; whereas in our case (of probable obstruction to the pancreatic duct) the fæces contained 143·80 grms. of fat, *i.e.*, 73·05 per cent. So that 26·95 per cent. of the fat given was absorbed from the intestines, and rendered capable of being made use of in metabolism.

The 2 cases seem fair ones to compare, as, in both, the quantity of food given was about equal; the only difference being that, while the boy received a larger quantity of fat in his diet, Rübner's received a larger quantity of nitrogen.

Turning now from what the results given in these tables show as regards the absorption to the actual nourishment, it may be as well to express it in the form of calories; that is to say, the quantity of heat necessary to raise 1 kilo., 1° C.

From Rübner's² calculations we obtain the following results:—

¹ Rübner, *Ztschr. f. Biol.*, München, 1889, bd. xv. s. 115.

² Rübner, *ibid.*, 1893-5, bds. xix. and xxi.

1 grm. proteid converted into urea, uric acid, ammonia, etc.	= 4.1 calories.
1 „ fat converted into carbonic acid and water	= 9.3 „
1 „ carbohydrate converted „ „ „	= 4.1 „

In metabolism experiments it is customary to reckon that 100 grms. of proteid contain on an average 16 per cent. of nitrogen, so that if we multiply the quantity of nitrogen by 6.25 we get the quantity of proteid it represents.

In our own case we did not estimate the quantity of carbohydrates, so that an average of other analyses has been taken.

If we convert the quantity of food given to our patient into calories we find—

Proteid, . . .	82.51 × 4.1 =	338.25 calories.
Fat, . . .	195.85 × 9.3 =	1830.71 „
Carbohydrate, .	198.75 × 4.1 =	814.87 „
		<hr/>
		2983.83 „

Consequently, the patient had received in his diet 2983.83 calories, and as he weighs 37.4 kilogs. (78 lbs.) during the days of observation, he received 78.9 calories per kilog. in his food.

Numerous observers have found that a normal man on an average requires from 30 to 40 calories per kilog. to maintain his weight, according to the amount of muscular work, and that 32 calories per kilog. is sufficient for most people doing an ordinary amount of muscular exercise. Thus, then, our boy received in his diet, twice the quantity of nourishment necessary to maintain his body weight, yet notwithstanding this he lost flesh.

The loss of weight is partly explained by the greatly diminished absorption of food which we have found by analysis of the fæces to have occurred, and we therefore see the importance of both analysing the urine and fæces before formulating a diagnosis in cases like his.

If we now subtract from the quantity of food given the quantity which we have found by analysis to have remained unabsorbed from the alimentary canal, we get the following results. The carbohydrates in our case not having been calculated, I have taken the results found by Abelmänn as my standard. He found in partial extirpation of the pancreas that only 77 to 78 per cent. of carbohydrate given in the food was absorbed from the intestines.

In the case of the boy we find—

	Given.	Unabsorbed.	Absorbed.	
Proteid, . . .	82.5	— 32.75 =	49.72 × 4.71 =	203.85 calories.
Fat, . . .	196.85	— 143.80 =	53.05 × 9.3 =	493.37 „
Carbohydrates,	198.75	— 43.00 =	155.75 × 4.1 =	638.58 „
			<hr/>	
				1336.80 „

From these calculations it is seen that, instead of the boy absorbing into his system 2983.83 calories, he really absorbed 1336.80 calories; that is to say, only 36 calories per kilog.

On returning to Table III. we see that he weighed 37·8 kilogs. while on ordinary diet. On the first day of milk diet (14th December) his weight remained the same, while on the third day (15th December) it fell to 37·6 kilogs., and on the fourth day (16th December), to 37·4 kilogs., and remained so on the 17th. Whereas on resuming ordinary diet (on the 18th) it rose to 37·6, and, on the 19th, to 37·8 kilogs.

The results of our analysis have shown that during his milk diet, while taking a large quantity of food, he only absorbed a small part of it, but at the same time he absorbed as much as 36 calories per kilog., and in spite of this he lost weight. From which it appears since 32 to 34 calories per kilog. per diem would have been ample for him to keep up his body weight in health, it was insufficient under the circumstances.

We must therefore conclude that not only was there in his case a disordered absorption of food from the alimentary canal, but there was also a defective metabolism of what was absorbed. Having shown that in the case of the boy there is not only a diminished absorption of both fat and proteids from the intestines, but that there is also a defective assimilation of the food materials actually absorbed, I will now turn to the chemical changes which the fat has undergone in its passage along the alimentary tract.

The following table shows the changes milk fat undergoes during its passage through the alimentary canal in the case of the boy under observation :—

TABLE V.—*Showing the Composition of the Fat in the Fæces of a Boy suffering from probable Obstruction of the Pancreatic Duct, and the Composition of Fat in the Milk given.*

	Total Fat.	Neutral Fat.		Free Fat Acids.		Fat Acids as Soap.		Cholesterin.	
		Total.	Per Cent.	Total.	Per Cent.	Total.	Per Cent.	Total.	Per Cent.
Milk, . .	196·85	191·000	97·02	5·690	2·89	0·121	0·06	0·160	0·08
Fæces, 16th,	149·72	59·051	39·44	54·348	36·30	26·270	17·55	10·051	6·71
„ 17th.	137·87	49·149	35·65	61·355	44·50	18·135	13·15	9·231	6·70

In Table V. we find the quantity of neutral fat taken has diminished from 191 grms. to 59·051 and 49·149 grms. respectively, so that a large quantity of it has either been absorbed during its transit along the alimentary canal, or, in spite of the absence of the pancreatic secretion, it has been broken up into fat acids, etc.

In the milk given there was only 5·690 grms. of free fat acids, while 54·348 and 61·355 grms. were found in the fæces. From this it is seen that we can at least account for the disappearance of part of the neutral fat from the alimentary canal, by its having been split up

into free fat acids in its passage along the intestines, seeing that they were increased tenfold.

As regards soaps, their quantity in the milk was only 0·121, while in the fæces it was no less than 26·270 and 18·135 grms.

Thus, in spite of the pancreatic secretion being absent, the neutral fats have not only been split up into free fatty acids and glycerine, but the fat acids have been able to find an alkali wherewith to form soap.

The amount of cholesterin in the milk was only 0·16 grms., while the quantity found in the fæces was 10·051 and 9·231 grms. respectively. This increase cannot be regarded as being due to any chemical change in the milk, but is, in all probability, due to a quantity of cholesterin being eliminated by the bile, or due to intestinal secretion.

That the above chemical changes should have taken place in the milk fats during their sojourn in the alimentary canal, in the boy, might be argued against the absence of the pancreatic juice really occurring; but experiments have shown me that the same thing occurs when we have undoubtedly not only hindered the flow of pancreatic juice into the intestines, but have removed the entire gland.

I here give a table showing the results found in the above boy, and those I found in the fæces of dogs which had had their pancreas extirpated:—

TABLE VI.—*Showing average Composition of Fat in the Fæces of a normal Dog on Milk diet, compared with one from which the Pancreas had been removed, placed side by side with the average result obtained in the two days' analysis in the case of the Boy supposed to be suffering from obstruction of the Pancreatic Duct.*

	Total Fat.	Neutral Fat.	Free Fat Acids.	Soap as Free Fat Acids.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Normal dogs,	100	34·17	58·65	7·19
Pancreas extirpated, . . .	100	33·90	55·25	10·84
Boy with obstructed pancreatic duct,	100	37·55	40·40	15·35

It is seen in Table VI. that if we take the total ether extract of the fæces as representing 100, the quantity of neutral fat contained in it is, in normal dogs, 34·17, while in those from whom the pancreas was artificially removed, as well as in the boy, we get respectively 33·90 and 37·55 per cent.

In the normal dogs, while the free fat acids are 58·65 per cent., in dogs without the pancreas, and in the boy, they are respectively 55·25 and 40·40 per cent. On the other hand, the soaps as represented as free fat acids are, in normal dogs, only 7·19 per cent., while, after pancreatic extirpation, they are increased to 10·84 per cent., and in the

case of the boy they are 15·35 per cent. Consequently, these cases very closely resemble each other in so far as neutral fat and fat acids are concerned. In fact, merely from the analysis of neutral fat and fat acids, one would be unable to say whether the pancreatic juice was present or absent. In the case of the soaps, we see that there is a slight tendency to excessive formation, or, I should rather say, an excessive excretion of soap in the stools when the pancreatic juice is hindered from reaching the intestines. This may be regarded as most remarkable, since when the pancreas is either entirely removed or its secretion is merely prevented reaching the alimentary canal, there is either a non-absorption, or a greatly diminished absorption of fat from the intestines.

According to former ideas, this non-absorption would have been attributed to the fat splitting-up action of the pancreatic juice no longer coming into play, and from the fats not being broken down, no emulsification taking place, and therefore no absorption.

In the case of the dogs, as well as the boy, it is seen from the above analysis that, in spite of the absence of this fat-splitting ferment of the pancreas, the fats during their passage through the alimentary canal are, by some means or another, broken up, and not only form free fat acids, but also soaps. Yet, in spite of this fact, they are not absorbed.

Abelmann¹ showed that when the pancreatic juice was prevented reaching the intestines, the ether extract of his dog's fæces contained even as much as 80 per cent. as free fat acids, and only a small portion as soap. Hédon and Ville² showed that when bile was prevented from entering the intestines by ligature of the common bile duct, the fæces of a dog contained 41 per cent. of soap, 57 per cent. of free fat acids, and only 2 per cent. of neutral fat. He then removed the greater part of the pancreas, only the tail being left to prevent glycosuria; the fæces now contained 45 per cent. of the ether extract as fat acid, 55 per cent. as neutral fat, but no soap, milk having been used as the diet.

I am still investigating the subject of the splitting up of neutral fat in the alimentary canal, and will therefore at present not go further into the question. One important fact is, that in spite of the absence of pancreatic juice, fat is able to be split up in the alimentary tract.

It may be well to try and explain what is the probable state of affairs in the case of the boy we have been investigating.

The results of the analyses show us that large quantities of fat appear in the stools, no less than 73·05 per cent. of the total quantity given. And, still further, the proteids excreted in the fæces are far above the amounts normally found, so that 40 per cent. of the nitrogen given has been excreted in the stools.

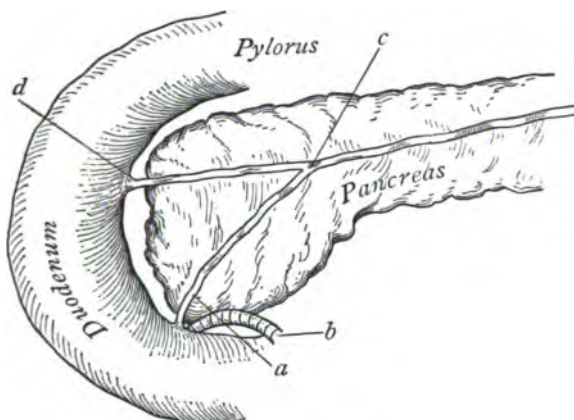
The foul odour of the stools was a constant feature in the case of

¹ Abelmann, *loc. cit.*

² Hédon and Ville, *Compt. rend. Soc. de biol.*, Paris, 1892, p. 308.

my dogs, and was, at the same time, specially marked in the case of this boy, so that it is worthy of note. These facts led me to believe that for some reason or other the pancreatic secretion was not reaching the alimentary canal. That this is not due to an absence of the pancreas, or destruction by disease, is shown by the fact that sugar at no time has been present in the urine, and, therefore, the evidence is in favour of an occlusion of the duct of the pancreas.

The morbid anatomical condition which has led to this obstruction would appear to be some chronic inflammatory stricture of the duct. At the same time, since analysis has shown us that bile is reaching the intestine, the common bile duct must have remained free. We cannot suppose that the common orifice of the bile and pancreatic duct can even be partially obstructed, for since bile and pancreatic juice are secreted at about the same pressure, namely, 200 mm. of water, it is hardly possible that only one should be hindered reaching the intestines. The pancreas is, however, known to have, very frequently, accessory ducts, or in some rare cases to enter the duodenum separately from the common bile duct.



- a.* Main pancreatic duct.
- b.* Common bile duct.
- c.* Point of junction of main and accessory pancreatic duct.
- d.* Accessory pancreatic duct.

The commonest form of accessory duct is one entering the duodenum nearer the stomach *d*, and we know from the history of the case that the boy suffered from gastritis previous to the appearance of the foul-smelling motions. It is conceivable that either the main duct of the pancreas *a* may be absent, and only the accessory duct is present, so that the inflammation spreading from the stomach down the duodenum may have involved it, without having extended far enough to have likewise involved the orifice of the common bile duct *c*. In this way there might have been a complete obstruction to the pancreatic duct, or we can imagine that both the main duct *a* and the accessory duct *d*

were present, but the inflammation had extended up the accessory duct so as to involve also the main duct.

This being the diagnosis, and it being impossible to give a drug which could with any certainty cure the morbid condition, it was necessary to consider what could be done in the case.

Abelmann found that feeding dogs after removal of the pancreas with raw pancreas caused an increased absorption of fat. I therefore recommended Dr. Auld to give the boy raw pancreas. From 28th February until 10th March 1895 this was done; and during this time the quantity of oil passed with the stools was very markedly decreased, although it did not entirely disappear. The foul smell was absent, but, since the boy was at the same time taking calomel and potassium benzoate, both powerful intestinal antiseptics, we can hardly put this fact down to the raw pancreas. The parents, later, refused to continue the pancreas treatment, in consequence of his having been ill after eating a supposed bad one. Since this date oil has been present, off and on, intermittently, and Dr. Auld has endeavoured to get the parents to allow the boy to resume taking the pancreas. During the last 4 months, with careful dieting and treating symptoms, he has increased 2 lbs. in weight.

In conclusion, it may be said, from the results of the analyses in this boy's case, and of the dogs' cases from which the pancreas was either partially or completely removed, that the pathology of the absence of pancreatic juice from the intestines is more complicated than one is at first led to believe, seeing that not alone is there a diminished absorption of fat, so that only 26.95 per cent. of the total given was absorbed in the case of the boy, and from 4 to 37 per cent. in dogs; but, at the same time, the proteid absorption is greatly diminished, so that in dogs only 18 per cent., and in the boy 60 per cent., was absorbed.

The results of the analysis of the faeces in the boy has shown that the non-absorption of fat after removal of the pancreas is not due, as is generally supposed, to any want of the splitting up of the neutral fat into fat acids, glycerine, and the formation of soaps, but, on the contrary, is due to some cause as yet unexplainable.

When the quantity of food given is increased above the quantity necessary for a healthy individual, so that the quantity absorbed may be equal to the number of calories necessary to maintain the body weight in health, owing to improper metabolism, they are insufficient to keep the weight up to the normal standard when the pancreatic duct is obstructed. On still further increasing the quantity of food, however, the body weight can be maintained, and we see that in the case of this boy careful dieting, together with treatment, has not only been able to keep up his weight, but even to cause a gain of 2 lbs. in the space of 4 months.

THE ACTION OF TOLUYLENEDIAMIN : A CONTRIBUTION TO THE PATHOLOGY OF JAUNDICE.

PART I.

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(PLATE XVII.)

THE action of toluylenediamin has formed the subject of an extensive series of observations by Stadelmann; and this has been supplemented in various directions by Afanassiew, Noël Paton, and Engel and Kiener.

Stadelmann's observations relate to the nature of the jaundice produced by this drug, and more particularly to the intimate changes in the bile to which the jaundice is due.

Afanassiew's observations relate more particularly to its destructive action on the blood, and to the vascular changes it produces in the liver.

Noël Paton¹ made use of the drug in his observations regarding the relation of urea formation to destruction of blood.

Engel and Kiener² have studied more particularly the pigment changes in various organs following its destructive action.

The author's observations have related more especially to the action of the substance on the blood, the distribution of its destructive action in various parts of the circulation and in various organs, the channels by which it is excreted, and its action on the liver and on the bile ducts and duodenum in the course of its excretion.

The latter observations bear very directly on the pathology of the jaundice caused by this drug; and with these it is proposed to deal in this first part.

I. STADELMANN'S OBSERVATIONS.

In their entirety the researches of Stadelmann constitute the most important contributions to the pathology of jaundice that have been made within recent years.³

¹ *Journ. Anat. and Physiol.*, London, 1886.

² *Compt. rend. Acad. d. sc.*, Paris, 1887, p. 465.

³ "Das Toluylenediamin und seine Wirkung auf den Thierkörper," *Arch. f. exper.*

Their chief result is to show that a number of forms of jaundice, formerly regarded as of non-obstructive and hæmatogenous nature, are really of obstructive origin.

The result has been arrived at from a careful study of the changes undergone by the bile in forms of jaundice connected with an excessive destruction of blood.

Toluylenediamin poisoning.—Of special interest and importance in this relation has proved to be the study of one drug in particular—toluylenediamin.

This drug has the peculiar action, first noted by Schmiedeberg, of causing intense jaundice in dogs. Stadelmann, who, at Schmiedeberg's suggestion, undertook to investigate its action, was at first quite unable to explain the jaundice. He failed to find any evidence of duodenal catarrh; nor at first did he find that it had any destructive action on the blood.

Its action, however, occasions well-marked changes in the bile. In the first stage the bile is increased in quantity, and is very rich in bile pigments; this stage beginning about 2 hours after the injection, and lasting about 12 hours.

Then follows a second stage, during which it loses all the characters of bile, and is replaced by a small quantity of viscid, colourless mucus. This begins about the fourteenth hour, and lasts from 60–70 hours, after which the bile gradually returns to its normal character.

The jaundice begins during the first stage, reaches its maximum during the second, and gradually disappears during the third.

The jaundice is marked by the presence of bile acids in the urine, sometimes in abundant quantity. This increase of the bile acid does not occur contemporaneously with the increase in the bile pigments. On the contrary, they are usually diminished during the first stage, at the time when the bile pigments are increased. It is only about the twenty-second, thirty-first, or forty-eighth hour that they appear in the urine; they reach their maximum in the next 24 hours, diminish during the following 24 hours, and then disappear altogether.

Their appearance in the urine, therefore, does not correspond in point of time with the development of the jaundice, for the latter is well developed 15–20 hours after the injection, while the bile acids do not appear until later.

In one important particular Afanassiew¹ supplemented these observations by showing that the drug exercises a markedly destructive action

Path. u. Pharmakol., Leipzig, 1881, bd. xiv.; "Zur Kenntniss der Gallenfarbstoffbildung," *Ibid.*, Leipzig, 1882, bd. xv.; "Arsenwasserstoffvergiftung: Ein weiterer Beitrag zur Lehre vom Icterus," *Ibid.*, Leipzig, 1883, bd. xvi.; "Das Chronische Vergiftung mit Toluylenediamin," *Ibid.*, Leipzig, 1887; "Weitere Beiträge zur Lehre vom Icterus," *Deutsches Arch. f. klin. Med.*, Leipzig, bd. xviii.; "Ueber den Einfluss des experimentell in den Körper Eingeführten Hämoglobins auf Secretion und Zusammensetzung der Galle," *Ibid.*, Leipzig, bd. xxvii.; "Der Icterus und seine Verschiedene Formen." Stuttgart, 1891.

¹ *Ztschr. f. klin. Med.*, Berlin, bd. vi.

on the blood, an observation subsequently confirmed by Stadelmann himself.

This observation seemed to supply the missing clue to the explanation of the jaundice. That explanation, according to Stadelmann, is that the drug causes a destruction of blood; the hæmoglobin set free leads to an increased formation and excretion of bile pigments; this increased excretion is attended by an altered character of the bile in the direction of increased viscosity; this, in the face of the low pressure at which the bile is excreted, causes a temporary obstruction, and leads to a reabsorption of the bile; and when the action of the drug exhausts itself, the bile gradually loses its viscid character, the flow of bile is re-established, and the jaundice disappears.

In the case of this drug, then, it will be noticed that a jaundice which at first sight has all the character of a hæmotogenous jaundice depends upon intimate changes in the character of the bile, leading to a temporary arrest of its flow.

Phosphorus poisoning.—And similarly with a well-known form of jaundice, always regarded as a typical example of non-obstructive jaundice—phosphorus poisoning has been shown by Stadelmann to be due to similar changes, differing only in one respect, that they are slower in their production.

Ten hours after the administration of this poison, the bile begins to be darker in colour; the bile pigments are increased by one-half, the bile acids at the same time being diminished. For the next 24 hours these conditions persist, and no jaundice develops.

Then the bile begins to change its character; it becomes clearer, more mucoid; its quantity sinks to about one-fifth; the bile pigments fall to one-half or one-third their normal amount; and the bile acids fall to a very low amount, 0·1, 0·15, or 0·7, instead of the normal 1·96.

It is at this stage that jaundice develops; but it only reaches a maximum about 5 days after the administration of the poison.

As the jaundice disappears there is again an increased excretion of bile pigments, doubtless derived by reabsorption from the tissues.

The bile acids, however, remain diminished for some days longer; and it is not till the tenth or eleventh day that they again reach their normal.

Arseniuretted hydrogen.—A similar explanation applies to the jaundice occasionally produced by inhalation of arseniuretted hydrogen.

Stadelmann's observations show that the action of this is attended by a remarkable condition of concentration of the bile, the gall-bladder and bile ducts being filled with a thick viscid bile, which frequently contains large quantities of amorphous sediment, as well as numerous crystals of bilirubin. Hence he concludes that the jaundice is undoubtedly the result of absorption—hepatogenous and not hæmatogenous.

The increase in the bile pigments in the first instance was as great

as three and a half times its previous amount; and relative to the quantity of bile excreted it was still greater (twenty times), the quantity of bile being reduced five and a half times.

The bile acids, on the other hand, were reduced to as much as one-tenth their normal amount.

The relation of the various events to each other he conceives to be as follows:—"Without doubt the breaking down of the blood is the occasion for the icterus—but only through the agency of the liver, which produces an abnormal bile, in consequence of the abnormal blood conveyed to it."

II. CONSIDERATION OF FOREGOING RESULTS.

These observations are important, not only as serving to explain the action of the particular drugs, but still more in relation to the pathology of so-called "non-obstructive" jaundice generally.

The jaundice of phosphorus poisoning has long been held to be a striking example of jaundice unconnected with any obstruction—of a jaundice due to suppression. So also the jaundice connected with an excessive destruction of hæmoglobin, *e.g.* in burns and scalds (Klebs, Ponfick, Lassar); following the injection of water or hæmoglobin (Kühne, Tarchanoff); or the inhalation of ether and chloroform (Nothnagel); of poisoning with pyrogallie acid or naphthol (Neisser); of paroxysmal hæmoglobinuria, malaria, pernicious anæmia—has been held to be an example of a jaundice of hæmatogenous origin.

And yet for phosphorus itself, as well as for toluylenediamin and arseniuretted hydrogen, which both exercise a markedly destructive action on the blood, these observations would appear to show that the jaundice they severally occasion, differing as it does in respect of its intensity, rapidity of onset, and other characters, is in all cases the result of changes in the character of the bile, and is essentially obstructive in its nature.

Moreover, the very feature which was held to distinguish a jaundice of hæmatogenous from one of *obstructive* origin, namely, the absence of bile acids from the urine in the former, receives from these observations an entirely different explanation.

It has hitherto been assumed that the formation of bile pigments and bile acids by the liver cells must necessarily go hand in hand, and that an increase or diminution in the one must be accompanied by a corresponding increase or diminution in the other. On this assumption, the absence of bile acids from the urine at any time when bile pigments were present, was regarded as denoting that the bile pigments had been formed elsewhere than in the liver. Stadelmann's observations demonstrate for the first time that the two processes do not necessarily go hand in hand. On the contrary, a large excretion of bile pigments may be attended by a diminished excretion of bile acids, and conversely.

In no respect, indeed, are his observations, in my opinion, more striking than in the demonstration they afford of this fact; and Stadelmann himself attaches a special importance to it.

If then, as would appear, bile pigments can be formed by the liver cells without any corresponding formation of bile acids, the absence of bile acids from the urine in certain forms of jaundice need not necessarily denote, as has hitherto been supposed, inactivity on the part of the liver. On the contrary, it is quite compatible with increased activity on the part of that organ in transforming hæmoglobin into bile pigments, and with a jaundice of purely obstructive nature connected with the excretion of these bile pigments.

On this view, then, many forms of jaundice connected with disorder of the blood, hitherto regarded as of non-obstructive nature, must be grouped as varieties of obstructive jaundice—the occasion of the obstruction being intimate changes in the character and consistence of the bile, similar to those caused in phosphorus and toluylenediamin poisoning.

Stadelmann's Explanation of the Obstruction.

An important point, then, with regard to such forms of jaundice, is to understand how the blood changes are related to those in the bile which occasion the obstruction.

According to Stadelmann, the relationship is of this kind. The blood disorder is usually marked by increased destruction of blood; the increased supply of hæmoglobin to the liver occasions an increased formation of bile pigments, which is attended at the same time by an increased concentration of the bile, caused by the specific action of the poison on the liver cells; this change in the character of the bile suffices to cause temporary obstruction to the flow of bile (with jaundice). The most constant and important change he regards as the increased formation of bile pigments (polychromia), so much so, that in his opinion the jaundice might most fitly be termed "jaundice from polychromia."¹

He takes strong exception to the term "polycholia" in this relation, as fitly describing the character of the jaundice. He contends that the term polycholia can only be rightly applied where there is an increase of all the constituents of the bile, both watery and solid. And, as he points out, this is not the case in the forms of jaundice now under consideration. So far from the quantity being increased, it is often diminished from the very first; and so far from the bile salts being increased, they are as a rule notably diminished, only regaining the normal when the jaundice is passing off.

III. AUTHOR'S OBSERVATIONS.

It is on this point, as to the relation between the blood changes and the bile changes, that my observations yield information.

¹ Stadelmann, "Der Icterus und seine Verschiedene Formen," p. 247.

They relate to the action of toluylenediamin, and go to show that the concentration of bile, so marked a feature of its action, is due to an extensive catarrh of the bile ducts, extending from their origin downwards towards the duodenum, occasioned by the excretion of the poison or its derivatives through the bile.

It is the increase of the catarrhal viscid mucus thus occasioned that causes the concentration of the bile, and finally arrests for the time being its flow altogether.

Action of Toluylenediamin on Duodenum and Bile Ducts.

EXPERIMENTS.

EXPERIMENT 63.—SMALL DOG ; weight, 4400 grammes.

Nov. 24, 1887.—0·5 gramme toluylenediamin in neutral saline solution injected subcutaneously.

Nov. 26.—Jaundice of conjunctivæ well marked, also evident in skin of ears and mucous membrane of gums.

Nov. 28.—Weight 4300 ; jaundice very pronounced all over body, especially seen over abdomen.

Nov. 29.—Jaundice still pronounced ; weight, 4050. Animal killed with chloroform.

Liver.—Greatly jaundiced, large, soft, and somewhat fatty looking.

Gall bladder.—Contains a quantity of extremely dark, thick, inspissated bile.

Periphery of lobule presents a bright, golden-yellow appearance, seen on microscopic examination to be due to the presence of large masses of yellow material. Many of even the larger bile ducts plugged with inspissated bile.

Great stasis of bile also in bile capillaries in centre of lobule.

Intralobular blood capillaries filled with coloured remains of hæmoglobin and red corpuscles.

Stomach.—Firmly contracted ; empty ; mucous membrane thrown into rugæ, of a pale yellow colour.

Duodenum.—Appearances very striking. From the pylorus downwards, for a distance of about 12 inches, its mucous membrane is extremely swollen and congested, showing at parts small hæmorrhages. This is most marked at the upper part of the duodenum, especially around the opening of the common duct.

The congestion and swelling of mucous membrane diminish in intensity from this point downwards, and disappears altogether about the commencement of the jejunum.

On comparing with the mucous membrane from the duodenum of a healthy dog, the mucous membrane is seen to be soft and swollen, and on section twice to three times as thick. Small hæmorrhages are seen in its substance.

EXPERIMENT 64.—FOX-TERRIER DOG ; weight, 7·15 kilogs.

Dec. 2, 1887.—Spleen excised with full antiseptic precautions ; edges of abdominal incision brought together with catgut ; skin edges with silk.

Dec. 5.—Animal recovered ; weight, 6450 ; 0·5 gramme toluylenediamin injected subcutaneously.

Dec. 6.—Conjunctival jaundice.

Dec. 7.—Pronounced jaundice all over body.

Dec. 8.—Jaundice still more marked; weight, 6050.

Killed with chloroform.

Peritoneal wound healed.

No inflammation.

Liver.—Very dark and full of blood. Lobules not distinguishable to naked eye. Microscopically, the central part of each lobule presents the most beautiful natural injection of the bile capillaries with bile.

Gall bladder.—Contains a small quantity of intensely dark bile.

Stomach.—Mucous membrane normal, pyloric end bile-stained.

Duodenum.—Contains a large quantity of deeply bile-stained viscid mucus. The mucous membrane is greatly swollen, soft, and congested, with here and there punctiform hæmorrhages. The congestion is most intense at the upper part around the opening of the common bile duct, and diminishes gradually from there downwards, till at a distance of some 10–12 inches from the pylorus the mucous membrane assumes a normal healthy appearance. The intestinal mucous membrane throughout is covered with deeply bile-stained thick viscid mucus.

The appearances are similar in character to those presented in foregoing experiment, only less marked.

EXPERIMENT 121.—Nov. 27, 1889. SMALL DOG; weight, 4·75 kilogs.; 0·3 grammes toluylenediamin injected subcutaneously.

Nov. 26.—100 c.c. dark bilious-looking urine.

Nov. 27.—Weight, 4·5 kilogs.; well-marked jaundice; 160 c.c. urine, deeply bile stained.

Nov. 28.—Weight 4·35 kilogs.

Jaundice very pronounced.

Killed with chloroform.

Stomach.—Empty; mucous membrane thrown into folds, normal in colour and appearance; towards pyloric end slightly bile-stained.

Duodenum.—The mucous membrane of duodenum is congested and ecchymosed, presenting marked injection at parts. The congestion and ecchymosis commences on a level with the papilla on which the bile duct opens; and is very marked over a tract of 3 inches in extent from this papilla downwards.

Just below the papilla the congestion has a circular arrangement around a central deeper and paler part, the appearance at first glance resembling that of an ulcer. The floor, consisting of follicular tissue, is pale; around the margins the villi are swollen, congested, and ecchymosed. At the upper part three such congested follicular patches—axis transverse to that of gut—measuring 13 mm. by 4 mm., 10 mm. by 5 mm., the third incomplete. One inch lower down another similarly congested patch, 11 mm. by 4½ mm.; and 1½ inches still lower down, another, 11 mm. by 7 mm.

The congestion extends from here the whole way down to the ileo-cæcal valve, without, however, any ecchymoses; the mucous membrane swollen and œdematous. Just above the ileo-cæcal valve, over an area of 3½ inches in extent, there is a repetition of the redness, swelling, and ecchymosis seen at the upper part of the duodenum. Near lower end of ileum, follicular patches prominent and swollen, but not reddened. At this point a considerable quantity of dark brown bile, sufficient to fill the lumen of the gut; elsewhere throughout the gut, deeply bile-stained mucus.

The whole wall of the gut is affected by the swelling, most of all, however, the mucous membrane.

A number of measurements made on a healthy dog give an average thick-

ness of $2\frac{3}{4}$ mm. for wall of gut; the measurements here give a thickness of $4\frac{3}{4}$ –5 mm., the mucous membrane alone being $3\frac{3}{4}$ mm. thick.

EXPERIMENT 129.—Dec. 7, 1889. Dog; weight, 5·5 kilogs.; 0·25 gramme toluylenediamin injected subcutaneously.

- Dec. 8.—80 c.c. clear straw-coloured urine.
No bile pigments.
Gives marked reaction of toluylenediamin.
- Dec. 9.—Urine slightly darker in colour.
Still no bile pigments.
Reaction of toluylenediamin distinct but less marked.
- Dec. 10.—Distinct reaction of bile pigments in urine.
No jaundice of skin or conjunctivæ.
- Dec. 11.—*Urine*—Distinct reaction of bile pigments.
No reaction of toluylenediamin.
0·34 gramme toluylenediamin subcutaneously.
- Dec. 17.—Weight, 4·55. No jaundice.
Killed with chloroform.

Duodenum and intestine.—On opening intestine from below upwards, mucous membrane is seen to be of pale colour and healthy appearance up to within 12–18 inches of pylorus. It then shows slight traces of congestion, but these are not marked, and are not such as would attract attention.

EXPERIMENT 133.—Dec. 10, 1889; weight, 10·9 kilogs.; 0·5 gramme toluylenediamin injected subcutaneously.

- Dec. 14.—10·1 kilogs.
- Dec. 15.—0·5 gramme injected.
- Dec. 16.—9·25 kilogs.
- Dec. 17.—8·95 kilogs.
Killed with chloroform.

Extreme jaundice from second day. During whole time the animal took no food and rapidly emaciated.

Stomach.—Empty. Mucous membrane thrown into folds, pale and normal.

Duodenum.—Even on opening abdomen, the duodenum seen to be affected, its walls turgid, swollen, and doughy to the touch. On being opened, the most intense inflammatory congestion of its coats, especially the mucous and submucous coats; the lumen of the gut narrowed, and filled with clear, thick mucus free from bile. The congestion and swelling has its greatest intensity around the opening of the common bile duct, from which thick mucus can be seen issuing; and for a distance of about 8 inches downwards from the point it is of the most intense character, the whole walls greatly œdematous and thickened.

Below this it gradually fades off, but throughout the whole of the small intestine the mucous membrane is considerably swollen and congested, the follicular patches here and there being very prominent by the congestion around their margins.

The appearances are accurately represented in the accompanying Plate, from a drawing made immediately on opening the duodenum (see Plate XVII.).

Summary of Foregoing.

In all these cases then, following the injection of toluylenediamin, there was found a condition of congestion and catarrh of the mucous

membrane of the duodenum, commencing on a level with the opening of the bile ducts, and always having its greatest intensity around these openings. The degree of congestion varied in different cases. In only one case was it of so slight a nature that it might, perhaps, have been overlooked, and that was the only case in which there was no jaundice. In all the other cases the condition of congestion was very marked. In the last, so great was the swelling of mucous and submucous coats, that the lumen of the duodenum was greatly lessened, the contents being clear, colourless, viscid mucus, free from bile.

In respect of its intensity, the condition in this last experiment was exceptionally striking. The attempt to produce it was purposely made. My object was not merely to produce extreme jaundice, but to see whether, by giving another large dose (0.5 gramme) while the jaundice was still very marked, the duodenal condition could be correspondingly intensified. The result was that all the essential features were intensified, especially the limitation of the congestion upwards, and its great intensity around the opening of the bile ducts. In addition, however, in this case, the catarrh of the bile ducts was very manifest—the clear viscid mucus projecting from the orifice of the common bile duct being of the same character as that occupying the duodenum itself.

As regards the relation of this duodenal condition to the jaundice, it will be noted that in only one case was there little or no obvious jaundice (although bile pigments were present in the urine); and that was the only case in which the duodenal condition was so slight that it might conceivably have been overlooked (Exp. 129). In the other cases the swelling and œdema of the mucous membrane was such that the effect of such a condition, if extending to the bile channels, could not but be obvious. The animals were all deeply jaundiced. A condition of catarrh falling far short of that here produced would amply suffice to retard, and, if continued, finally arrest the flow of bile along the bile channels.

The catarrhal condition was, however, not confined to the duodenum. From the swelling of the papilla on which the bile duct opened, and the presence in the orifice of the duct of colourless mucus similar to that lying within the duodenum; from the circumstance, further, that in all cases alike the congestion and swelling had a maximum intensity around the bile duct openings, it seemed that whatever had occasioned the condition had reached the duodenum through the bile.

To the duodenal condition *per se*, as a factor in producing the obstruction to the flow of bile by extension upwards of the catarrh into the common duct, I attach little or no importance. But as an indication and visible evidence of the degree of inflammatory catarrh that can be set up in a mucous surface by the action of an irritant, I attach to it special importance.

It is to be noted that the drug was not administered by the mouth, but hypodermically; that the mucous membrane of the stomach above

the pylorus was always free from the slightest trace of abnormality or congestion; and that precisely on a level with the opening of the bile duct the mucous membrane of the duodenum had undergone this extensive swelling, oedema, and congestion. The irritant—using that term in its widest sense—appeared to have reached the duodenum through the bile; and its action on the mucous membrane of the bile duct, judging from the similarity in character of the colourless mucus issuing from the bile duct, had been the same as on the mucous membrane of the duodenum.

IV. EXCRETION OF TOLUYLENEDIAMIN THROUGH THE BILE.

I made the following experiments to ascertain whether the poison was excreted through the liver, and could be found in the bile:—

EXPERIMENT 119.—Nov. 19, 1889. Dog; weight, 10·15 kilogs.

Narcotised with morphia (0·15 gramme).

Small dose of curare given.

Right ureter exposed, and a cannula introduced.

Common bile duct exposed, and a glass cannula introduced.

Gall bladder found distended with richly-coloured *bile* (bilirubin colour).

11.45 A.M.—Operation completed.

12 noon.—Bile flowing fairly well; some hindrance to flow of urine.

12.7 „ —Still no flow of urine; 20 c.c. warm normal saline solution injected into vein.

1 P.M.—3·5 c.c. of urine }
3 c.c. of bile } = Sample A.

10 c.c. blood withdrawn from femoral artery = I.

1.30 „ —1·6 c.c. urine }
2·3 c.c. bile } = Sample B.

1.35 „ —0·5 gramme toluylenediamin in 20 c.c. normal saline injected into jugular vein.

2 „ —Secretion of urine has been very slow.

1·9 c.c. urine }
2·2 c.c. bile } = Sample C.

2.33 „ —2 c.c. bile = Sample D.

3 „ —1·4 c.c. bile = Sample E.

2·2 c.c. urine = Sample D. and E.

The latter muddier and distinctly darker in colour than first specimen.

5 c.c. of blood withdrawn into saline solution = Sample II.

3.30 „ —2 c.c. bile = Sample F.

Bile secretion during last half-hour has been greater in quantity; colour of bile is less than in Sample E.

No flow of urine.

3.55 „ —20 c.c. of $\frac{3}{4}$ per cent. NaCl solution injected into vein.

3.56 „ —Urine again flowing.

4 „ —1·1 c.c. bile = Sample G.

0·6 c.c. urine.

4.35 „ —5 c.c. blood withdrawn = III.

5.30 „ —2·7 c.c. bile.

1·4 c.c. urine.

5.35 P.M.—5 c.c. blood withdrawn = IV.

5.45 „ —4 c.c. bile = Sample H.

1.4 c.c. urine = Sample H.

Urine from bladder obtained by squeezing.

Experiment stopped.

Animal killed by bleeding from femoral artery.

Blood of a dark chocolate colour.

I. Greater portion withdrawn into a beaker containing $\frac{3}{4}$ per cent. NaCl solution.

II. Remainder into 8 per cent. NaCl solution.

Placed below in cold cellar.

Weather at the time cold.

Nov. 20, 10 A.M.—I. Has firmly coagulated. A large quantity of serum expressed.

A portion of this serum carefully drawn off with a pipette. No trace of blood-colouring matter either to naked eye or on spectroscopic examination. No hæmoglobin.

II. Supernatant serum quite colourless. No trace of hæmoglobin on spectroscopic examination.

Examination of Bile for Toluylenediamin.

Method.—The method I employed was a colorimetric one, first suggested to me by the phenylenediamin test for nitrites in water. I found that the process could be reversed. Toluylenediamin, even in extremely dilute solution, gives a very well-marked reddish-brown colour reaction on adding to the solution a drop of dilute hydrochloric acid, and subsequently a drop or two of a solution of nitrite of sodium.

I had at first some difficulty in applying the test in the case of coloured fluids, like bile, urine, blood.

After a number of experiments, with the object of decolorisation, *e.g.* with charcoal, heating, etc., the best method, I found, was to dilute the urine and bile greatly, and to use as the comparison solution one of normal bile and urine diluted to the same colour. Thus, in the case of bile, the method was as follows:—

1. A few drops of the bile placed in a test tube and diluted up to a certain point.

2. In another test tube a small quantity of normal bile diluted till the colour was the same as in 1.

On applying the test to the latter the yellow colour entirely disappeared and solution remained colourless.

If toluylenediamin were present, even in minute trace, a faint brownish coloration was seen on looking through a deep layer of the fluid; and this became quite marked when more was present.

The solutions were placed in cylindrical, flat-bottomed glasses of uniform diameter, and the degree of coloration was judged by looking down through the fluids against a background of white paper on which the glasses stood.

In the foregoing experiment the results, as regards the bile, were as follows:—

Samples A. and B.—Taken before the injection of toluylenediamin. No reaction.

Sample C.—Twenty-five minutes after the injection. No reaction.

„ D.—One hour after injection. Merest trace of characteristic colour reaction.

„ E.—One and a half hours. Still very faint, but more marked than before.

„ F.—Two hours. Reaction easily appreciable; even in thin layers.

„ G.—Two and a half hours. Still more marked.

„ H.—Four hours. Diluted up to 28 c.c. Of this, 2½ c.c. taken and diluted, and tested. Its yellow colour gives place to a distinct brownish colour. A control solution of normal dogs' bile similarly diluted and tested, yellow colour completely disappears. No trace of reaction in this latter.

Quantitative Estimation of Toluylenediamin in Bile.

The method I employed was to ascertain what quantity of a standard solution of toluylenediamin was necessary when added to normal bile to produce the same degree of colour reaction as the bile under examination.

The following was the standard solution:—

Either 0·3 mgrm. of toluylenediamin in 100 c.c. water, or 1 mgrm. in 100 c.c.

The last specimen of bile—4 hours after injection—gave a colour reaction equivalent to 0·47 mgrm. of toluylenediamin.

Examination of Blood withdrawn during Experiment.

1. Withdrawn before the injection.
Gives no reaction of toluylenediamin.
2. Three hours after injection.
Gives a trace of reaction.

Examination of Blood after Death.

PORTION I.—Withdrawn into $\frac{3}{4}$ per cent. NaCl solution.

Both serum and clot give distinct reaction of toluylenediamin.

The method employed was as follows:—

Clot beaten up in water, raised to boiling point, and filtered. The filtrate treated with equal parts of methylated spirit, and again filtered.

A colourless solution then obtained.

Serum raised to boiling point and filtered.¹

Treated in this way—the serum contained an estimated quantity of 0·6 mgrm.

Serum.—Total quantity, 60 c.c.

2 c.c. gave distinct colour reaction, equivalent to colour reaction in water of 0·02 mgrm.

60 c.c. = 0·6 mgrm.

Clot.—Total quantity of fluid, 70 c.c.

2 c.c. = Distinct reaction.

70 c.c. = 0·7 mgrm.

Total quantity of toluylenediamin in blood, 1·3 mgrms.

¹ I subsequently found it better not to heat the serum, but to test it pure, using healthy serum as the control solution.

Examination of Urine.

SAMPLES D and E.—(2·2 c.c.) One and a half hours after injection. Distinct reaction of toluylenediamin.

Diluted to 30 c.c.

2 c.c. contains 0·05 mgrm.

30 c.c. „ 0·75 „

SAMPLE H.—(1·4 c.c.) Four hours after injection.

Diluted to 60 c.c.

2 c.c. requires 7 c.c. of standard solution.

(1 mgrm. in 100 c.c.) of toluylenediamin = 0·07 mgrm.

60 c.c. = 2·10 mgrm.

Urine of bladder diluted up to 50 c.c.

2 c.c. required 1·3 c.c. of standard solution = 0·13 mgrm.

50 c.c. = 3·25 mgrms.

Total found in urine = 6·10 mgrms., giving an excretion of 2·85 mgrms. from left kidney, and 3·25 mgrms. from right kidney.

In this experiment there was definite evidence of an excretion of the poison through the bile as early as one hour after its injection, and by the fourth it was in appreciable quantity (0·47 mgrm.).

At the same time it was being excreted in the urine—the total quantity excreted in 4 hours being 6·10 mgrms.

V. EXCRETION OF TOLUYLENEDIAMIN THROUGH THE URINE.

EXPERIMENT 121.—Dog; 4·75 kilogs. in weight.

Nov. 25.—0·3 gramme toluylenediamin subcutaneously.

Nov. 26.—100 c.c. dark bilious urine.

Whole carefully collected, amounting with washings to 150 c.c.

3 c.c. when diluted gave colour reaction equal to 3·4 c.c. of standard solution of toluylenediamin. (10 mgrms. in 100 c.c.)

3 c.c. = 0·34 mgrm.

150 c.c. = 17 mgrms. (0·017 gramme).

Nov. 27.—Urine dark bilious. With washings, 230 c.c.

No trace of toluylenediamin in reaction.

Only one-eighteenth part of the amount injected accounted for in the urine; the remainder broken up within the body.

EXPERIMENT 125.—Dog.

Dec. 2.—0·4 gramme injected.

Killed by bleeding $1\frac{1}{4}$ hours later; the blood withdrawn into 200 c.c. $\frac{3}{4}$ per cent. NaCl solution.

Blood.—Twenty-four hours later firmly coagulated; the serum colourless, showing no trace of hæmoglobin bands.

Both serum and clot (the latter after being treated with distilled water and boiled) gave a slight reaction of toluylenediamin; the total being estimated as not more than 1 mgrm.

Duodenum.—Mucous membrane covered with thick dark bile for a distance of 6 inches, and slightly congested. Mucus and bile scraped off, diluted with water, heated, and filtered. Filtrate gave faintest reaction.

The following experiments show the delicacy of the test employed:—

EXPERIMENT 131.—RABBIT bled into 50 c.c. $\frac{3}{4}$ per cent. NaCl solution, to which 10 mgrms. of toluylenediamin had been added.

Serum pipetted off; quantity, 100 c.c.

2 c.c. gave colour reaction equivalent to 0.19 mgrm.

100 c.c. = 9.5 mgrms.

Blood clot gave no reaction.

Total accounted for, 9.5 mgrms = (95 per cent.).

EXPERIMENT 132.—RABBIT bled into 50 c.c., 10 per cent. NaCl solution, to which 10 mgrms. of toluylenediamin had been added.

Serum carefully pipetted off; gives marked reaction of toluylenediamin.

Total quantity, 100 c.c.

2 c.c. gave colour reaction equal to 0.195 mgrm.

100 c.c. gave colour reaction equal to 9.75 mgrms.

Total accounted for 9.75 out of 10 mgrms. injected = 97.5 per cent.

VI. SUMMARY OF FOREGOING.

The foregoing experiments may be regarded as definitely establishing that the bile is one of the channels through which toluylenediamin is excreted from the body.

As early as 1 hour after injection traces of it could be detected in the bile (Exp. 119 and Exp. 125); and by the fourth hour it was present in an appreciable, albeit still very small, amount.

In the present relation, however, the actual amount excreted is a matter of comparatively little importance. The point of importance, as it appears to me, is that it is excreted at all through this channel.

The other experiments I have recorded show that toluylenediamin is broken up very soon after its introduction into the body; and that the greater part of it is either destroyed, or leaves the body in some other form. Information on this point is afforded most clearly by Experiment 121, in which of the amount of toluylenediamin injected (0.3 gramme), only about $\frac{1}{8}$ th part could be discovered in the urine (0.17 gramme).

No less striking in the same relation is the small amount of the poison to be detected in the blood even 1 hour after its injection. Thus $1\frac{1}{4}$ hours after injection of 0.4 gramme (Exp. 125), only a trace could be found in the whole quantity of blood.

It may be, of course, that the poison has already entered into some combination, *e.g.* with hæmoglobin, which prevents its presence being detectable. It certainly is not present in free form; for the Experiments 131 and 132 show that the method employed for detecting it is sufficiently delicate to recognise 95 to 97 per cent. of the substance when added to blood outside the body.

Considering, then, the small amount present at any time in the

blood, and the small proportion ($\frac{1}{8}$) that succeeds in escaping unbroken from the body, the trace found in the bile is not inconsiderable.

The question then arises, Are the catarrh and congestion set up in the bile ducts and duodenum, after the injection of toluylenediamin, to be ascribed to the irritant action of this small trace of poison in the bile?

I think this extremely improbable, and for this reason. Toluylenediamin has a slight irritant action, especially in its stronger solution ($2\frac{1}{2}$ or 5 per cent.) when injected subcutaneously, causing some inflammation, with subsequent necrosis of overlying skin and scab formation.

Such an action, however, is, I consider, no criterion of what its action on the mucous surfaces of the bile ducts or duodenum may be. I think it extremely improbable that in the small amount in which it is present in the bile, it can exert such an irritant action as that I have described.

It is, I conceive, much more likely that the irritant action of the bile after toluylenediamin poisoning is due to the presence of derivatives of the poison, and not to the poison itself. The foregoing observations show that only a small proportion ($\frac{1}{8}$, Exp. 121) passes through the body unchanged. The remainder is either destroyed, or converted into derivatives, which, like the poison itself, are in all probability excreted through the urine and the bile.

One reason that inclines me to believe that it is to the products, rather than to the poison itself, that the irritant action is to be ascribed, is the following.

I have not found any trace of duodenal catarrh in rabbits and cats after administration of toluylenediamin. And yet any irritant action it possesses on subcutaneous injection is as marked, according to my experiments, in rabbits as in dogs.

Why, then, does the effect of its action on the duodenum differ in the two cases? It is, I think, more likely that this difference is due to a difference of metabolism. Toluylenediamin is much better borne by rabbits than by dogs.

The important point is, that, *whatever the nature of the irritant*, these observations show that *it is contained in the excreted bile*; that *it reaches the duodenum through the bile*, and that *it must be of powerful nature to induce such an inflammatory congestion as that described*.

Cause of the Jaundice.

The explanation of the jaundice produced by toluylenediamin appears to me, then, to be—that products of the poison are excreted through the bile, and excite swelling and catarrh, with increased secretion of mucus, in its course down the bile passages into the duodenum.

The involvement of the duodenum I do not consider at all necessary

for the production of the jaundice. It is a mere adjunct to the catarrh of the bile ducts. It is only in exceptionally severe cases that it occurs. The primary condition in causing the obstruction is, I consider, the catarrh of the bile ducts at their origin. Under the low pressure at which the bile is excreted, a slight degree of catarrh in this situation suffices to cause, first, a retardation in the flow and increased consistence of the bile (end of first stage), and as the catarrh spreads down the bile ducts, it finally arrests its flow altogether (second stage).

This view, as to the relation of the catarrh to jaundice, differs essentially, I would point out, from the one usually accepted,¹ according to which the catarrh spreads upwards from the duodenum. The latter must always be preceded by duodenal catarrh. In the present case, as I have said, I consider the duodenal condition quite unnecessary to the production of the catarrh. For the course of the catarrh is from above downwards; it commences in the bile radicles, and extends downwards; and in many cases it may not even reach the duodenum. As it is produced by substances excreted by the liver, it involves, therefore, the whole bile radicles and smaller ducts simultaneously, and to a like degree. In slight cases it may not extend beyond these; and it would be quite impossible (apart from its result, jaundice) to recognise it after death.

As a factor in causing jaundice, it derives its chief importance from the widespread character of the obstruction it produces, rather than from its high degree. The increased secretion of mucus occasions increased viscosity of bile sufficient to occasion a certain amount of obstruction and a certain degree of jaundice.

VII. COMPARISON OF FOREGOING WITH STADELMANN'S RESULTS.

Stadelmann² was never able to observe any obstruction to the free exit of bile into the duodenum. And if there could be any doubt on this point, he regarded his experiments on dogs with biliary fistulæ as conclusive.

"If jaundice could be equally well produced in them, the jaundice could not be catarrhal—the result of catarrh or inflammation of the duodenum extending up into the ductus choledochus."

I have analysed Stadelmann's original experiments on which he bases the above conclusions, and I here present the result.

His first two experiments are of no value in the present relation, as the animals were not killed.

EXPERIMENT 3.—LARGE DOG; 20 kilogs. in weight; 0·4 gramme hypodermically.

Next day.—No jaundice; 0·4 gramme more.

Following morning killed; indistinct icterus of conjunctiva.

¹ Murchison, "Diseases of the Liver," 3rd edition, 1885, p. 159.

² *Loc. cit.*, p. 120.

Sectio.—Liver somewhat icteric, but by no means so markedly as in other cases. Bile ducts, gall bladder, common bile duct, full of bile and considerably distended. Common duct opens free into duodenum, and "on gentle pressure" bile can be squeezed out of it.

Small bile ducts extraordinarily full of bile. Contents of intestine much bile-stained.

EXPERIMENT 4.—20 kilogs.; 1·0 gramme hypodermically; found dead and cold next morning.

Ductus choledochus free.

No injection of intestine.

Liver only in parts icteric; gall bladder full, and also bile ducts. On cut surface of liver "bile flows freely out of bile ducts."

Intestinal contents strongly bile-stained.

EXPERIMENT 5.—5 kilogs.; 0·5 gramme hypodermically; next morning found dead, although still warm.

No jaundice of conjunctivæ or mucous membrane.

Result same as in foregoing.

EXPERIMENT 6.—MIDDLE-SIZED DOG; 0·3 gramme hypodermically; hæmoglobin in urine.

No jaundice over 7 days later.

0·2 into jugular vein.

Two days later no jaundice.

Then 0·3 into vein of foot.

Next day.—Urine jaundiced, but no blood; dog no longer observed.

EXPERIMENT 7.—5 kilogs.; biliary fistula without ligaturing common duct.

Eight days later 0·2 *per os*.

Two days later well-marked jaundice, which increased from day to day.

(How long after not stated) Dog killed.

"No sign of catarrh" in intestine.

Intestinal contents strongly bile-stained.

Common bile duct pervious to a probe.

"On pressure over the upper part of it," bile flowed into intestine.

No mucous plug anywhere discoverable in bile ducts.

EXPERIMENT 8.—SMALL DOG; 0·15 gramme by mouth.

Two days later marked jaundice.

Two days later, *i.e.* on fourth day, found dead.

Sectio.—No signs of catarrh of mucous membrane of intestine; large quantity of bile in intestine; gall bladder quite full; bile ducts also full; "on gentle pressure" over the gall bladder, bile flows into intestine.

EXPERIMENT 9.—0·25 gramme hypodermically; slight jaundice, which gradually faded away from fourth day onwards.

At end of 8 days experiment broken off.

Four days later 0·3 gramme hypodermically.

Two days later another 0·3 gramme.

Three days later—so ill that it can hardly stand; killed.

Sectio.—Moderate icterus; gall bladder filled with green bile, whose exit into intestine is free; no signs of a catarrh of intestine; contents of intestine green-coloured.

Out of these nine experiments, three are valueless for our present purpose, as the animals were not killed (Exp. 1, 2, 6).

On comparing the remaining six with those recorded by myself, one difference is noticeable, sufficient of itself to account in no fewer than three cases for the absence of any duodenal condition comparable to that I have described. This difference is that in my experiments the animals, with one single exception, were always killed when the jaundice was at its greatest height; while in the foregoing experiments of Stadelmann the animals in two cases (Exp. 4, 5) died within 24 hours of acute poisoning, before any jaundice had developed; and in a third (Exp. 3), there was only a doubtful jaundice of conjunctiva at the time of death.

That, under these circumstances, no pronounced changes were found in bile ducts or duodenum, need not surprise, since as a matter of fact there was no complete obstruction.

Nevertheless there was, I consider, in all three cases evidence of commencing obstruction along the course of the bile ducts. For in all there was great stagnation of bile in the bile ducts, especially the smaller, notwithstanding the fact that the common duct opened free into the duodenum. There was clearly, therefore, no obstruction in the duodenum or at the mouth of the duct; and if, as Stadelmann thought, catarrh could only originate in the duodenum, and spread upwards into the bile ducts, the jaundice could not be catarrhal.

But the experiments do not justify the conclusion that there was no obstruction to the passage of bile into the duodenum, and no catarrh of the bile ducts. Obstruction, I think, there evidently was all along the bile ducts, since, notwithstanding that they were distended, and that the opening of the bile duct into the duodenum was free, "gentle pressure" was in all cases necessary to make the bile flow into the duodenum.

In the remaining three experiments there was a similar absence of any duodenal condition. "On pressure" bile could always be made to pass into the duodenum; or a probe could be passed through the common bile duct (Exp. 7). It would, I think, be fallacious, however, to regard this as proof of the absence of any obstruction of catarrhal nature.

With regard to this last test, I do not doubt that in the worst case of duodenal inflammation and catarrh among my experiments, I should have been able to pass a probe through the bile duct had I attempted it. For, after all, the obstruction is only caused by thick viscid mucus, and catarrhal swelling of the epithelium of the bile ducts, and that is not in itself sufficient to resist a probe, however effective it may be in stopping the flow of bile.

The further point to which Stadelmann attaches importance in favour

of the non-catarrhal origin of the jaundice is: the occurrence of jaundice "equally well" in dogs with biliary fistulæ—in whom, therefore, the bile flows freely through a fistula in the gall bladder, and its flow is independent of any condition of the duodenum or the common bile duct (Exp. 7).

On this I need hardly dwell, since I have shown that the catarrh of the bile ducts produced by this substance is altogether independent of the duodenum, and spreads downwards, not upwards. Such a catarrh is not likely to be affected by the presence of a biliary fistula, except in so far as the common bile duct is thereby cut off, and the length of the biliary tract likely to be affected by it correspondingly shortened.

As a matter of fact, Stadelmann's experiments clearly show that jaundice is not "equally well" produced in dogs with biliary fistulæ as in normal dogs. On the contrary, as he himself admits, larger doses were always required in the former than in the latter to produce a similar degree of jaundice, precisely as one might expect from a shortening of the length of duct affected by the catarrh.

The foregoing observations and conclusions relate, in their entirety, to the jaundice of toluylenediamin poisoning.

They show that in catarrh set up by the irritant action of the poison on the mucous lining of the bile ducts from their origin downwards, we have an efficient cause for the increased viscosity of bile and obstruction to its flow.

As we have seen, the two special factors in bringing about the increased viscosity of bile are, according to Stadelmann (1) the increased formation of bile pigments (polychromia), and (2) the specific action of the poison on the liver cell.

VIII. RELATION OF JAUNDICE TO HÆMOGLOBINÆMIA.

It is necessary to consider what is the influence of an increased formation of bile pigments (polychromia) *per se*, on the consistence of the bile, and through the latter on jaundice.

That some relation exists between these two conditions is evident from the frequency with which some degree of jaundice is met with, both clinically and experimentally in cases where hæmoglobin has been set free in the blood and passes into the urine.

The observation that some relation exists between the two conditions formed in Kühne's hands the very starting-point of the doctrine of a hæmatogenous jaundice independent of any obstruction.

Kühne¹ considered this relation an absolute and quantitative one. An excess of free hæmoglobin in the blood was of itself sufficient, according to him, to occasion a certain degree of jaundice—such a degree at least as to cause bile pigments to appear in the urine.

¹ "Lehrbuch der physiol. Chemie," 1868.

The later experiments of Tarchanoff and Stadelmann seemed to lend additional support to this view.

Thus, according to Tarchanoff,¹ bile pigment is found regularly in the urine of dogs after injection of water or hæmoglobin into the blood.

And, as we have seen, Stadelmann attaches quite a special importance to the increase of bile pigments (polychromia) which occurs under such circumstances, as one of the chief factors in occasioning a form of jaundice. After injection of large quantities of hæmoglobin, he usually found a trace of bile pigment in the urine some time or other in the course of the experiment.

Stadelmann's observations go still further; for they show in what manner an excess of free hæmoglobin may produce jaundice. The injection of free hæmoglobin into the blood, or its liberation within the circulation by use of distilled water, is followed by changes in the bile, namely, increase of bile pigments, increased viscosity of the bile, diminution of bile acids—changes, therefore, similar in character to those caused by toluylenediamin or arseniuretted hydrogen. And the explanation of the jaundice that may occur under such circumstances is, according to him, the same, namely, obstruction caused by the high concentration of the bile, which is, in turn, related to the polychromia. Nothing at first sight seems wanting, therefore, to an understanding of the relation between hæmoglobinæmia and jaundice.

And yet other data, to which I must now direct attention, appear to me to indicate that the relation is by no means so simple and so constant as the above would appear to show. *They justify rather the conclusion that hæmoglobinæmia per se, apart from the operation of other factors, is not sufficient to cause jaundice.*

Here, however, I must guard myself against a possible misconception. I am not here disputing that there is the closest relation between certain forms of jaundice and increased destruction of blood (including, in some cases, hæmoglobinæmia). On this point both clinical and experimental observations are at one; and the subject is one to which I shall have presently to refer. What I am here endeavouring to ascertain is the influence of a simple excess of free hæmoglobin in the blood in producing jaundice, apart from all other possible factors.

Hæmoglobinæmia is not of itself sufficient to cause bile pigments to appear in the urine or jaundice.

And, first of all, I would direct attention to observations throwing doubt on the accuracy of the statement that excess of free hæmoglobin in the blood is sufficient of itself to cause bile pigments to appear in the urine.

Results in support of such a view have only been obtained in one class of animals (dogs), and even in them not constantly; whereas in rabbits the balance of experimental evidence from all sides is, I consider, decidedly against the view that hæmoglobinæmia *per se* is sufficient to cause jaundice.

¹ *Arch. f. d. ges. Physiol.*, Bonn, bd. ix.

Dogs.—As regards dogs, the significance to be attached to bile pigments in the urine is a little complicated by the circumstance, noted by many others, which I can fully confirm, that it is not uncommon to find a trace of bile pigments in the urine of apparently healthy dogs. Unless this source of fallacy be kept in mind, and care be taken by suitable precautions to avoid it, erroneous conclusions may be drawn. In my experience, this is specially likely to be the case in old dogs. To avoid this source of fallacy it is, I think, necessary to employ only young healthy dogs, whose intestinal canal has been well cleared out by castor oil or calomel; the animals being kept on a milk-and-bread diet for some days previous to experiment. This tendency to the occurrence of a slight degree of icterus in dogs is connected, in my opinion, with their habit of eating garbage and refuse of various kinds. It is not uncommon to find in such animals some degree of catarrh of the mucous membrane of the small intestine.

The experiments of Naunyn¹ conclusively show that if care be taken to avoid the above source of fallacy, hæmoglobinæmia even in dogs does not necessarily cause bile pigments to appear in the urine. In only two out of six cases in which he caused hæmoglobinuria by injecting hæmoglobin subcutaneously, did the urine give any reaction to Gmelin's test for bile pigments; and in both of these cases the urine had given a slight reaction before the experiment. In the other four cases he failed to find any bile pigment in the urine, although there was marked hæmoglobinuria (and necessarily hæmoglobinæmia).

And although Stadelmann usually found a trace of bile pigment in the urine after injection of large quantities of hæmoglobin, there are, as it appears to me, certain features in his results which indicate that the presence of bile pigment was to be referred to other factors than the simple hæmoglobinæmia. Thus the quantity was at most a trace, and in no way proportionate either to the amount of hæmoglobin set free or to the resulting increase of bile pigments. This is not what one would naturally expect, if the presence of bile pigment in the urine be directly related to the hæmoglobinæmia, as Kühne supposed, or to the resulting increased formation of bile pigments (polychromia), as Stadelmann would have us believe.

Thus in one experiment (*a*) of the latter, where there was an increase of 80 per cent. in the bilirubin secreted, along with hæmoglobinuria, only a trace of bile pigment was found in the urine. While in another (Exp. 3), where the increase in bilirubin was less than 50 per cent., without hæmoglobinuria, there was a considerable quantity of bile pigment in the urine.

I have summarised the results of his experiments² in the following tables, for the purpose of bringing out the foregoing points. It will be seen from them that the one condition to which the jaundice is con-

¹ "Beiträge zur Lehre vom Icterus," *Arch. f. Anat. u. Physiol.*, 1868, p. 401.

² *Op cit.*, p. 23 *et seq.*

stantly related, is the degree of viscosity of the bile, not the degree of polychromia or hæmoglobinæmia.

EXPERIMENT (a).—Dog ; 20 grammes of hæmoglobin injected in six portions between 10 A.M. and 8 P.M.

First period of 12 hours—

Bilirubin increased by 80 per cent.

Hæmoglobin, but no bile pigment in urine.

Second period of 12 hours—

Bile very dark. Viscid, diminished in quantity.

No hæmoglobinuria, but a trace of bile pigment in urine.

Third period—

Bile increased in quantity.

No trace of bile pigment in urine.

Bile acids considerably diminished at the very time the bilirubin most increased.

EXPERIMENT 1.—10·02 grammes hæmoglobin injected subcutaneously.

First period of 24 hours—

Bilirubin increased by 56 per cent.

Bile reduced to one-third normal quantity, dark, viscid.

No bile pigment in urine.

Second period—

Bile very dark and thick.

Trace of bile pigment in urine.

Third period—

Bile normal in consistence.

No trace of bile pigments in urine.

Bile acids.—First 12 hours. Unaffected.

Second „ Diminished by 36·8 per cent.

Third „ „ „ 44·5 „

EXPERIMENT 2.—10·82 grammes hæmoglobin subcutaneously injected.

First period of 12 hours—

Bilirubin increased by 11·96 per cent.

Urine normal.

Second period—

Bilirubin increased by 61 per cent.

Bile thick.

Urine normal.

Third period—

Bilirubin increased by 35·8 per cent.

Bile of normal consistence.

Urine normal.

Quantity of bile fairly normal throughout.

Bile acids showed no change.

EXPERIMENT 3.—13·94 grammes hæmoglobin injected into peritoneum.

First period of 12 hours—

Bilirubin increased by 12·3 per cent.

Bile very thick ; quantity diminished.

No bile pigments in urine.

Second period—

Bilirubin increased 49·5 per cent.
 Bile thick ; quantity diminished.
 Considerable quantity of bile pigment in urine.

Third period—

Bilirubin increased by 12·4 per cent.
 Bile normal in quantity and consistence.

Bile acids. First and second periods—

Diminished by about 17 per cent.
 Third period—slightly increased.

The relation, then, between hæmoglobinæmia or polychromia and jaundice is no mere quantitative one even in dogs.

And if this be true of hæmoglobinæmia, produced by injection of hæmoglobin or of distilled water, it is still more true of hæmoglobinæmia induced by poisons.

Thus in one of the foregoing experiments of Stadelmann (Exp. 6), there was hæmoglobinuria after injection of 0·3 gramme toluylenediamin without any jaundice supervening ; and 9 days later, after a similar dose, there was jaundice without, however, any hæmoglobinuria.

Rabbits.—While then, as regards dogs, the evidence either way is inconclusive,—hæmoglobinæmia sometimes causing jaundice, sometimes not,—in rabbits there is no such uncertainty. The great preponderance of evidence is, I consider, decidedly against the view that hæmoglobinæmia *per se* suffices to cause jaundice, even in the slight degree necessary to cause bile pigments to appear in the urine.

Naunyn always failed to find bile pigments in the urine of rabbits after causing hæmoglobinuria.

Since then the list of those who have similarly failed includes the names of :—

Wickham Legg,¹ after the injection of bile acids.

Lauder Brunton,² after the injection of bile acids into the circulation, or of ether or dissolved blood corpuscles into the intestine.

L. Steiner,³ after the injection of water into the circulation of rabbits.

On this latter point my observations are in entire agreement with those of Naunyn and Steiner. Both after a slight and marked degree of hæmoglobinæmia, I failed to find bile pigments in the urine.

I append two experiments in point.

EXPERIMENTS 44 (Sept. 30, 1887).—RABBIT ; 30 c.c. of distilled water injected into jugular vein.

Slight hæmoglobinuria, with yellow granular débris of hæmoglobin, passing off in 5 hours.

No bile pigments detectable in urine with Gmelin's test.

¹ "Bile, Jaundice, and Bilious Diseases," 1880, p. 235.

² "Handbook for Physiological Laboratory," 1873, p. 499.

³ "Ueber die hæmatog. Bildung des Gallenfarbstoffes," *Arch. f. Anat. u. Physiol.*, 1873.

EXPERIMENT 48 (Oct. 10, 1887).—RABBIT; 70 c.c. distilled water injected into jugular vein.

Marked hæmoglobinuria; urine contained yellow granular débris of hæmoglobin in large quantity; also hæmoglobin casts; well-marked guaiacum reaction. *No bile pigments.*

In some cases the results have varied in the hands of the same observer. Thus Graham Brown,¹ after subcutaneous injection of bile acids in rabbits, failed in most cases to find bile pigments in the urine, while in a few cases he succeeded.

Naunyn, who failed to discover bile pigments in the urine when he injected hæmoglobin into the circulation, found it after injecting thawed blood into the intestine;² an observation which Lauder Brunton³ failed to confirm.

These varying results may perhaps be partly explained either on the ground that the animals used were not in all cases the same, or that the conditions of the experiments were not in all cases alike—different agents being used to produce the hæmoglobinuria, and different methods of administration employed. Hitherto, on the view that the positive result was the common and natural one, the chief endeavour on the part of observers has been to explain the negative results. But as I have shown, even in dogs the result is not always positive; and when it is, it is not directly related to the quantity of hæmoglobin set free, or the resulting formation of bile pigment. In rabbits, however, a positive result is decidedly the exception. The position of matters is thus, I consider, reversed. So long as it was held that free hæmoglobin could become transformed into bile pigment within the blood, it was natural to expect that hæmoglobinæmia should cause bile pigments to appear in the urine; and no less natural, therefore, to regard as exceptional all cases where this did not occur. Now, however, that it has been shown by preponderance of evidence that the jaundice of blood disorder is not hæmatogenous, but hepatogenous (Stadelmann), the position of matters is, as I have said, reversed. If under the old (hæmatogenous) view the difficulty was to explain why hæmoglobinæmia should not always occasion some degree of jaundice, with our present knowledge the difficulty appears to me of another nature—to explain, namely, in what way hæmoglobinæmia, with its resulting polychromia, should cause increased viscosity of the bile and temporary arrest of its flow.

For the reasons I have adduced then, I consider that mere excess of hæmoglobin in the blood, or increase of bile pigments, however great, is not the sole factor determining these changes in the bile, with the resulting jaundice.

Jaundice may be of the most intense character, with only a comparatively slight polychromia (one-half increase in toluylenediamin

¹ *Proc. Roy. Soc. Edin.*, 1875, p. 528.

² *Op. cit.*

³ *Op. cit.* p. 499.

poisoning), or it may be slight or absent, with a threefold or fourfold increase (arsenious acid poisoning).

IX. RELATION OF BLOOD DESTRUCTION TO JAUNDICE.

What then is the relation between increased destruction of blood and jaundice? As I have shown in the foregoing section, it is, in my view, no mere *quantitative* one. Simple excess of free hæmoglobin in the blood, even when in quantity sufficient to cause hæmoglobinuria, does not necessarily of itself suffice to cause jaundice.

That jaundice, however, frequently occurs in conditions attended by increased destruction of blood is a fact about which there can be no dispute. The relation between the two conditions must thus be a *qualitative* one, but of what nature is by no means clear. Thus, in one class of animals (dogs), toluylenediamin causes jaundice without hæmoglobinuria; in another (cats), it causes hæmoglobinuria without jaundice; while in a third (rabbits), it causes apparently neither hæmoglobinuria nor jaundice. And yet in all three classes it occasions a destruction of blood. Moreover, even in the same animal its action sometimes varies. Thus in the dog it may occasionally cause hæmoglobinuria without jaundice (Stadelmann).

Various explanations of these differences have been put forward. According to Afanassiew,¹ the occurrence of jaundice alone, or hæmoglobinuria alone, or both together, depends on the *extent* of the preceding blood destruction, and on its *nature*.

He distinguishes three kinds of action of hæmolytic substances on the blood. The first is represented by the action of glycerine, which dissolves the hæmoglobin out of the corpuscles, leaving hardly any morphological remains of the latter within the blood. The free hæmoglobin escapes mostly through the kidneys, only a small portion being dealt with elsewhere (liver, spleen, or bone marrow).

The second kind of action is represented by that of toluylenediamin. This acts quite differently. It breaks the red corpuscles into pieces. These circulate in the blood, and accumulate in the liver, spleen, and bone marrow, where they are disposed of, only a part escaping through the kidney. When the dose is small, no hæmoglobin passes into the plasma. Hence, in slight cases, only jaundice occurs, produced by the increased excretion of bile; there is no hæmoglobinuria.

When the dose is larger, the remains of the red corpuscles are not sufficiently rapidly disposed of; they circulate in the blood, some of their hæmoglobin escapes into the plasma, and not only jaundice, but also hæmoglobinuria occurs.

The third kind of action is represented by that of pyrogallie acid.

¹ "Ueber die pathologischen anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hæmoglobinurie oder Icterus verbundenen Vergiftungen," *Virchow's Archiv*, 1884, bd. xcvi. s. 465.

It is intermediate in character betwixt the two preceding. It liberates hæmoglobin from the corpuscles, and causes hæmoglobinuria; but morphological remains (Schatten, etc.) also soon appear, and there is usually slight icterus.

This explanation of the differences in the action of different substances on the blood is adopted by Silbermann.¹

Stadelmann² hesitates to accept it, but cannot altogether reject it. It may be sufficient, he thinks, to account for the jaundice, but it is quite insufficient, in his opinion, to explain the degree of jaundice. He considers that the blood corpuscles of different species of animals have a different resisting power towards different poisons; but any essential difference in the nature of the action of different poisons on the blood he could not discover. Individual differences there undoubtedly are. Thus in dogs, arseniuretted hydrogen causes intense hæmoglobinuria and well-marked changes in the blood (altered corpuscles, Schatten, etc.), while with toluylenediamin signs of blood destruction are infrequent. But this is not constant. Every now and again a case is met with in which the latter drug causes intense hæmoglobinuria without jaundice, although the morphological changes in the blood are the same as those which usually attend its action when it causes jaundice.

X. AUTHOR'S OBSERVATIONS.

My observations on this point relate to the action of distilled water, glycerine, pyrogallie acid, and toluylenediamin, but more particularly to the last.

With regard, first of all, to the modes of action of the above agents on the blood, my observations confirm in the main those of Afanassiew regarding the differences in the blood changes in the case of individual drugs.

I do not find, however, that these differences are of such a character, either in degree or in kind, as to account for the very different action of the agents qua the production of jaundice.

As regards *glycerine*, this agent is, in my opinion, unsuitable for comparison experiments with such agents as pyrogallie acid or toluylenediamin. When injected subcutaneously, as in Afanassiew's experiments, it produces intense inflammatory œdema; and much of the hæmoglobinæmia is due to its action on the red corpuscles at the seat of injection. The œdematous fluid is hæmoglobin-tinted.

A much more suitable agent of this class, I consider, is *distilled water* injected directly into the circulation. It likewise dissolves the hæmoglobin from the corpuscles, leaving apparently few morphological remains in the blood. At least this is the case, judged by the appearances presented in the circulating blood during life.

¹ "Ueber Hæmoglobinæmie," *Ztschr. f. klin. Med.*, Berlin, 1886, bd. xi. s. 471.

² *Op. cit.*, s. 237.

Few or no changes are presented in the circulating blood even after the injection of large quantities of distilled water (70 c.c., Exp. 48), notwithstanding that a great destruction of blood may have taken place. Thus, in Exp. 47, the hæmoglobin was reduced in amount by one-seventh in the course of 24 hours. In Exp. 48 there was intense hæmoglobinuria. If, however, the animal be killed within a few hours of the injection (Exp. 88), numerous stromata are found, especially in the capillaries of the liver (Exps. 47, 48, 88).

EXPERIMENT 47.—RABBIT; under observation for a week; red corpuscles, 5,650,000; hæmoglobin, 70 per cent.

Oct. 7. 1.30 P.M.—50 c.c. distilled water injected into jugular vein.

3 „ No débris, or schatten, or stromata in blood examined; pure.

Blood examined in $\frac{3}{4}$ per cent. NaCl shows a considerable quantity of granular débris, small colourless spherules; no schatten.

„ 8.—Red corpuscles, 5,480,000; hæmoglobin, 60 per cent.

Animal recovered, eating as usual.

Urine, 50 c.c.; large quantity of yellowish remains of hæmoglobin.

„ 10.—Red corpuscles, 5,290,000; hæmoglobin, 58 per cent.

Killed 3 days after operation.

Liver.—Cells fatty, especially in portal zone; free from blood pigment particles; no darkening in sulphide of ammonium.

Spleen.—Slight darkening in NH_4HS .

Bone marrow not affected by NH_4HS ; no excess of pigment.

EXPERIMENT 48.—RABBIT.

Oct. 10. 3 P.M.—70 c.c. distilled water injected into jugular vein.

4 „ No changes recognisable in corpuscles; no schatten; a little granular débris.

„ 11. 12 noon.—Red corpuscles show no abnormality; no granular débris; no schatten.

Urine contains large quantity of free hæmoglobin, and casts and granular remains of hæmoglobin.

„ 12. 11 A.M.—Killed (44 hours after operation).

Spleen considerably enlarged. A large number of red corpuscles present bud-like projections; a large quantity of colourless granules seen; detached buds are seen floating about, and also enclosed in splenic cells; distinct increase in blood pigment.

Liver.—Red corpuscles normal; no appearance of buds; liver cells fatty; no sign of blood pigment; no darkening in sulphide of ammonium.

Red bone marrow.—Blood corpuscles normal in appearance; no buds; a few pale stromata seen. Little or no darkening in NH_4HS .

EXPERIMENT 88.—RABBIT; 70 c.c. of distilled water injected into jugular vein; death 2 hours later; intense hæmoglobinuria.

Blood of portal vein.—Corpuscles pale, but no granular débris or obvious disintegration of corpuscles.

Splenic vein.—Corpuscles pale, but no granular débris or obvious disintegration of corpuscles.

Spleen.—Red corpuscles, for the most part apparently normal; a few show-

ing small bud-like projections; but little granular debris, and only a few pale colourless spherules.

Liver.—Marked polycholia; upper intestine filled with yellow bile; within capillaries great numbers of partially or completely decolorised stromata and colourless spherules.

In addition, very numerous small yellow spherules; not fat, resembling similar particles in the liver cells.

Spleen.—Contrast very great. Red corpuscles almost all normal, only a few showing small bud-like projections; very little granular debris, and only a few pale spherules.

After *pyrogallie acid*, large numbers of schatten are to be found in the blood if the dose be large (Exp. 58). At no time, however, are the number of schatten in the circulating blood at all proportional to the diminution in the number of red corpuscles. Moreover, with small doses, a large destruction may, I find, take place without any schatten formation at all, and with as little evidence of change in the blood as is found after injection of distilled water (Exp. 54).

EXPERIMENT 58.—RABBIT.

Date.	Weight.	Number of Red Corpuscles.	Changes in Red Corpuscles in Circulating Blood.	Remarks.
Nov. 11. 12 noon.	3050	6,280,000	Normal.	1 gramme (.33 per kilo) pyrogallie acid in 10 c.c. ($\frac{3}{4}$ per cent. NaCl) injected subcutaneously.
1.30 P.M.	...	5,950,000	Corpuscles quite normal.	...
5 P.M.	...	6,240,000	Corpuscles quite normal. Not a sign of blood destruction.	...
Nov. 12.	3050	6,300,000	Not a sign of blood destruction.	Animal depressed.
Nov. 14.	2850	5,790,000	Not a sign of blood destruction.	...
Nov. 16. 12 noon.	2850	5,890,000	...	1.5 gramme (0.52 per kilo) pyrogallie acid in 15 c.c. NaCl solution injected intravenously.
2.30 P.M.	...	5,560,000	Red corpuscles show no budding or fragmentation. No schatten.	...
5.30 P.M.	...	4,650,000	Small spherical fragments seen.	Circulation feeble, difficulty in getting blood.
Nov. 17.	2650	1,100,000	Extraordinary number of schatten, almost equalling in number the red corpuscles. The blood is dark in colour, watery in consistence. The absent corpuscles are by no means represented by their schatten. Great increase in number of leucocytes.	Animal apparently well. Urine 140 c.c., highly albuminous, and containing large quantity of remains of blood. No Gmelin's reaction.

EXPERIMENT 58.—RABBIT—continued.

Date.	Weight.	Number of Red Corpuscles.	Changes in Red Corpuscles in Circulating Blood.	Remarks.
Nov. 18.	2550	550,000	Blood very watery. Corpuscles form rouleaux imperfectly. Enormous increase in leucocytes—as many as fifty in a sparsely-filled field. Schatten in great number, although not so numerous as yesterday. Large quantity of granules; red corpuscles seen throwing off bud-like projections.	Animal looking very ill and anæmic.
Nov. 19.	2550	590,000	Blood very thin and watery; has lost its dark colour; shows a very large number of granules, and more marked alterations in red corpuscles. Schatten still to be seen, but much fewer in number. Leucocytes much fewer—fourteen in a field. A number of the red corpuscles are extremely large—pale, bi-concave—twice to three times larger than others. Some of them showing long processes (pear-shaped).	Very ill and feeble. Killed at 12.

EXPERIMENT 54.—RABBIT.

Date.	Number of Corpuscles.	Changes in Blood.	Remarks.
Nov. 4. 11.20 A.M.	5,350,000	None.	0.5 gramme pyrogallie acid in 10 c.c. NaCl solution, injected subcutaneously.
12.20 P.M.	5,090,000	No changes.	...
3.20 P.M.	5,150,000	No changes. No schatten.	...
Nov. 5.	4,240,000	No changes. No schatten.	...
Nov. 7.	4,400,000		Animal apparently in good health. Killed with ether.

Spleen becomes coal-black in sulphide of ammonium; contains a very large quantity of iron pigment.

Liver.—Very rich in blood; no blood pigment in cells; no darkening in NH_4HS .

After *toluylenediamin* poisoning there is less tendency to the formation of schatten, at least in dogs and rabbits. The red corpuscles seem rather to break up into yellow spherical particles—these may be seen in process of being thrown off as bud-like projections from the red corpuscles.

This process goes on especially in the spleen, according to my

observations. So much so indeed, that as I have elsewhere shown,¹ the removal of the spleen in rabbits diminishes, materially, the destructive action of toluylenediamin.

In cats, however, I find schatten formation is as marked a feature of toluylenediamin poisoning as it is of pyrogallie acid poisoning. Conversely, with pyrogallie acid I have found in some cases, along with formation of schatten, the red corpuscles throwing off buds in precisely the same way as is usually found after toluylenediamin (Exps. 45, 46, 55).

EXPERIMENT 45.—YOUNG RABBIT.

Sept. 29, 12.30.—0.25 gramme toluylenediamin in 5 c.c. $\frac{3}{4}$ per cent solution NaCl subcutaneously.

„ 30. —Animal unaffected; killed 12.30.

Liver.—A very distinct excess of fine granular blood pigment in cells, especially in portal zone, blackened with NH_4HS .

Spleen.—Very little darkening with NH_4HS ; no apparent increase of blood pigment.

Bone marrow of femur.—No abnormal appearances. Slight darkening in NH_4HS ; but no apparent increase of pigment microscopically.

EXPERIMENT 46.—YOUNG RABBIT.

Sept. 29, 12.30.—0.5 gramme toluylenediamin in 10 c.c. NaCl injected subcutaneously.

2.30.—Animal affected; looking rather ill, and lying down.

Blood shows no changes; no schatten or granules.

„ 30. —Purged during the night; depressed.

12.30.—Killed.

Liver.—Cells fatty; a very distinct excess of granular pigment; most of all in cells of portal zone; granules blackened by NH_4HS .

Spleen.—Pale and anæmic and contracted; shows a large number of yellow granules; little darkening in NH_4HS .

Bone marrow.—Slight over-darkening in NH_4HS ; microscopically, a number of yellow granules.

EXPERIMENT 55.

Date.	No. of Red Corpuscles.	Morphological Changes in Blood.
Nov. 10 . . .	6,000,000	None.
„ 12 . . .	6,110,000	„
„ 16 . . .	6,220,000	„
„ 16, 1 P.M. .	0.75 gramme toluylenediamin injected intravenously	„
„ 16, 3 P.M. .	6,105,000	One or two fading corpuscles seen; also few pale spherules.
„ 16, 6 P.M. .	6,080,000	No schatten; a considerable number of small spherical bodies seen, some throwing off bud-like processes.
„ 17, 11 A.M. .	4,970,000	Corpuscles perfectly normal in shape and appearance; no schatten; no granules.
Killed, 12 A.M.		

¹ *Lancet*, 1892, ii.

Spleen.—Not enlarged, or presenting any appearance of congestion. A considerable number of the red corpuscles show bud-like outgrowths, and many of these latter are seen floating about free as small yellow globules; no schatten.

Tissue becomes coal-black in sulphide of ammonium; microscopically, pigment in form of minute spherical granules of varying size, mostly free, but many lying within cells.

Liver.—Lobules mapped out by pale centres and congested margins. Cells are fatty; hardly any darkening in sulphide of ammonium.

Bone marrow.—No increase of pigment; no darkening in NH_4HS ; no appearance of budding in red corpuscles; a few small spheres, almost colourless, seen.

It will be noted that the chief evidence of the undoubted blood destruction (fall in the red corpuscles from 6,220,000 to 4,970,000 in 24 hours) was to be found in the spleen in the form of budding corpuscles, and great excess of blood pigment. No schatten were to be found.

Certain differences are thus observable in the *mode of action* of destructive agents on the blood corpuscles. But they are *not so distinctive either as regards their character or degree as to explain the remarkably different action of these drugs in the production of jaundice*. They are of some importance as serving to explain the occurrence or non-occurrence of hæmoglobinuria. But they serve in no way to explain the point at issue, namely, why in one case jaundice should occur where there is not even any hæmoglobinæmia, and is absent when the latter is pronounced.

The character of the blood changes not being sufficiently distinctive to account for the presence or absence of jaundice, we have to look elsewhere.

Another view suggests itself, namely, that the occurrence of jaundice may be influenced by the *character* of the hæmoglobin.

Lauder Brunton has suggested in the same relation that the differences observable in different animals may indicate differences in the relation of hæmoglobin to the liver cells in different classes of animals.¹

That individual differences exist in the character of the hæmoglobin of, *e.g.*, the dog, the cat, and the rabbit respectively, is, I think, exceedingly probable. The hæmoglobin of one has a poisonous action when injected into the other. But that these of themselves are sufficient to account for the differences as regards liability to jaundice in these animals is, I think, extremely improbable. Much more depends, in all probability, on the natural degree of concentration of the bile in these different animals. In the rabbit, *e.g.*, the bile is normally much more watery than in the dog, and its quantity is much greater. A factor, therefore, which in a dog might occasion such a degree of concentration of the bile as to lead to a temporary arrest in its flow, and to jaundice, might quite well be without any influence in the rabbit.

¹ Murchison's "Diseases of the Liver," 3rd edition, ed. by T. Lauder Brunton.

As a matter of observation, I have never noticed in rabbits any condition which could rightly be termed jaundice produced by action of destructive agents on the blood. Hence, as regards jaundice, comparisons between rabbits and dogs are inapt. The latter are as naturally subject to some degree of jaundice as the former are naturally immune; and this I consider arises not so much from any differences in the *character* of the hæmoglobin in the two cases, or in its *relation* to the liver cells, as from radical differences connected with the habits as regard food, etc., and metabolism of the two animals.

A third alternative suggests itself, namely, that the occurrence or non-occurrence of jaundice after an increased blood destruction may be dependent on the *form* in which the hæmoglobin is supplied to the liver. Thus, after the action of distilled water the hæmoglobin is liberated from the stroma of the corpuscle, and passes freely into the urine. After toluylenediamin the red corpuscles break up into yellow globules in a manner almost identical with their behaviour under the influence of high temperatures as described by Max Schultze.

That the hæmoglobin is supplied to the liver cells in different forms in these two cases is, I consider, certain from other evidence. It is the latter form of hæmoglobin that, according to my observation, most of all favours the formation of blood pigment both in liver cells and in spleen; and this, too, in different classes of animals alike—dog, rabbit, and pigeon, while, according to my observations, free hæmoglobin, such as is produced by distilled water, does not lead to the formation of blood pigment, either within liver cells or spleen (*vide Exps. antea*).

Under the action of toluylenediamin, then, the hæmoglobin does not, I conclude, become dissociated from the albuminous stroma of the corpuscle, as is the case with distilled water. It is found in the form of yellow droplets or spheres within the blood, within the spleen. In this form also it passes through the kidneys; not, however, as free hæmoglobin—for, notwithstanding the presence of much of this material in the urine, the urine gives none of the reactions of free hæmoglobin. There is no hæmoglobinuria in the ordinary sense of the term.

Similarly, in this form also the hæmoglobin reaches and passes into the liver cells.

It is, I think, important to note these physical peculiarities in the action of toluylenediamin on the blood corpuscles, and especially on the hæmoglobin of the blood. For although their bearing on the pathology of the jaundice caused by this drug may not be clear, they nevertheless may have some significance, as denoting changes of a more obscure chemical nature in the character of the hæmoglobin supplied to the liver in such cases. This at least, in my opinion, is the only possible direction in which the occurrence of jaundice may be affected by the *character* or *form* of the hæmoglobin supplied to the liver. But while admitting this, I do not consider it at all likely that such is the case. Nor do I consider that change in the character of the hæmoglobin is

sufficient to account for the changes in the bile which occasion the jaundice of toluylenediamin poisoning.

For the action of this poison on the blood is, according to my observations, identical in dogs and rabbits. Moreover, what is of more importance, is that in the rare cases in dogs, in which this drug causes hæmoglobinuria without jaundice, the changes in the blood are of the same character, differing only in degree, as those found when jaundice occurs without hæmoglobinuria (Stadelmann).

I consider, then, that some other factor than mere *quantity* or *character* of the hæmoglobin, must be responsible for the increased viscosity of bile produced by the drug.

And these being excluded as insufficient, there remains only to be considered one further possible factor—the influence, namely, of the poison itself—exerted not on the liver cells, as suggested by Stadelmann, nor on the blood, as supposed by Afanassiew, but on the biliary channels in course of its excretion, as I have shown.

So far as toluylenediamin itself is concerned, I consider that the effect of the catarrh resulting from its excretion (either of itself or of its products) in causing obstruction is fully established by the foregoing observations. And it is in this direction that the explanation of the differences in the behaviour of various blood-destroying agents, *qua* the production of jaundice, is, in my view, to be sought.

I have shown that neither hæmoglobinæmia nor (however intense) mere increase of bile pigments (polychromia) can account for the increased viscosity of bile which occasions the jaundice. The latter may be intense with only a half increase in the bile pigments (toluylenediamin), or only slight, possibly absent, with a threefold or fourfold increase (arsenious acid); or may be absent altogether with the intense hæmoglobinæmia occasioned by distilled water.

I have also shown that, in my opinion, change in the *character* of the hæmoglobin, although conceivably a possible factor, is not a probable one in bringing about the essential changes in the bile necessary for the production of obstruction, namely, the increase of viscosity. The only remaining factor, then, is the action of the poison that occasions the blood changes. According to the degree with which this is capable of exciting catarrh of the bile ducts, in course of its excretion, is there liability for the blood-destruction to be attended by jaundice.

XI. NOMENCLATURE.

The jaundice so produced is *hepatogenous*, inasmuch as it is due to obstruction occasioned by this increased viscosity of the bile. It may also be termed "*hæmo-hepatogenous*," as suggested by Afanassiew, inasmuch as it is preceded by changes in the blood. The term "*hæmatogenous*" has, however, been used in connection with jaundice in a special sense, as implying that the bile pigment itself is formed in the

blood, that its use in any other sense is apt to mislead. And hence the above term, although not inappropriate, is, in my view, open to objection on that ground.

To avoid this difficulty, Quincke has proposed the name *an-hepatogenous*, thereby denoting that while the jaundice is essentially hepatogenous, it is nevertheless closely related to, and dependent upon, other changes elsewhere (in the blood).

Stadelmann, as we have seen, regards the essential and most constant change as the increase of bile pigments; and the jaundice which results might, in his opinion, be mostly termed "*polychromic*, or "*jaundice from polychromia*."¹

I have endeavoured to show in the foregoing that this name is inappropriate, inasmuch as increase of bile pigments cannot be regarded as the essential change in the bile, or the chief factor in causing the obstruction.

So far as the bile pigments are concerned, identical changes, differing only in degree, are produced by agents so widely differing in their action *qua* jaundice, as normal saline (0·8 and 0·6 per cent.) solutions, salicylate of soda, distilled water, arseniuretted hydrogen, pyrogallie acid, phosphorus, and toluylenediamin. Thus normal saline solution (0·8 per cent.) may, as shown by Stadelmann, cause an increase of one-third in the bile pigments (bile acids being diminished to one-fifth or one-seventh)—changes, therefore, hardly less striking than the increase of one-half in the bile pigments (with bile acids reduced at first to one-half then to mere traces), which occurs in toluylenediamin poisoning; or the increase of one-half in the first 10 hours in phosphorus poisoning (with diminution in bile acids); or, lastly, the three-and-one-half-fold increase with arseniuretted hydrogen.

Clearly, then, a condition (polychromia) which is not proportional to the degree of obstruction cannot be regarded as the essential condition occasioning the obstruction. And that this is the case Stadelmann himself freely admits. While maintaining that the term polychromia best describes the jaundice, he admits that other factors must operate to bring about the change in the character of the bile itself on which the obstruction depends; and these factors are, in his view, to be sought for in a direct action of the several poisons on the liver cells, leading to a production of a highly concentrated bile.

It may, perhaps, seem a matter of comparatively little moment what name be given to this form of jaundice. I cannot, however, so regard it. The terms "*hæmatogenous*" and "*non-obstructive*," formerly and still in many cases applied to it, are no longer applicable, inasmuch as the jaundice is not hæmatogenous, but essentially hepatogenous and obstructive.

The term hepatogenous, therefore, fully describes its character anatomically. It is due to obstruction and to reabsorption of bile

¹ *Op. cit.* p. 247.

pigments, just as ordinary obstructive jaundice is. But it differs totally from the latter in its pathogeny; and some title is wanted to designate its relationship with the disorder of the blood with which it is ordinarily associated.

So far as toluylenediamin is concerned, the obstruction, as I have shown, is essentially of catarrhal origin; and the term "catarrhal jaundice" might, in my opinion, quite fitly and appropriately be given to it, but for one circumstance, namely, that this designation has come to be applied to, and reserved for, a form of jaundice of essentially different origin. Ordinary so-called catarrhal jaundice is, by a general consensus of opinion, supposed to arise from catarrh of the duodenum, involving the orifice of the bile duct secondarily. In no other sense, for example, is it spoken of by Murchison.

In the present case the course of the catarrh is quite a different one. It begins in the origin of the bile ducts, and extends downwards. If the duodenum is at all involved, and it is only in severe cases that it does become involved, it is only secondarily to the catarrh in the bile ducts.

It may perhaps be a question how far so-called catarrhal jaundice does arise in the way described, namely, secondarily to duodenal catarrh—to what extent it may not rather arise primarily in the bile ducts. In my opinion it is a very open question indeed. The latter I am disposed to regard as in all probability the common mode of origin of catarrh of the bile ducts.

But such a view is at variance with that commonly accepted, which regards catarrhal jaundice as arising secondarily to catarrh of the duodenum; and under these circumstances I do not consider that to apply the term "catarrhal" in a sense entirely different would be fitting.

Moreover, the term catarrhal, however appropriate, would not indicate the special feature of this variety of jaundice, namely, its relation to previous disorder of the blood.

That disorder, in the great majority of cases, is marked anatomically by increased destruction of blood (hæmolysis); and the term therefore which, in my opinion, would most fitly describe its character, would be that of "hæmolytic jaundice."

And yet even this term is not altogether free from objection.

It is true that increased hæmolysis accompanies and precedes in most cases the form of jaundice now under consideration, whether induced experimentally by destructive agents, such as water, ether, pyrogallie acid, toluylenediamin, etc., or occurring clinically in paroxysmal hæmoglobinuria, pernicious anæmia, malaria, yellow fever, icterus gravis, etc.

But, as I have shown, it is not the increased hæmolysis *per se*—with its hæmoglobinæmia or the associated polychromia—that can be held accountable for the jaundice; but rather is it that both the hæmolysis and the jaundice have one common factor.

The factor that occasions the catarrh on which the jaundice depends is, in my opinion, the poison; hence, on this view, the term which would in all respects most aptly describe this form of jaundice would, in my opinion, be the term "toxæmic."

It is free from the objection to which, as I have shown, the term *hæmo-hepatogenous* is open. It has the advantage over the term *hæmolytic*—which, in most respects, would be a very suitable one—in being, I consider, more generally accurate, and more closely indicative of the nature of the relationship between the jaundice and disorder of the blood. Lastly, it has the advantage of being not only appropriate as regards the pathology of the jaundice, but also as regards its features clinically, and the general clinical course it pursues. It is in toxic conditions generally—in pyæmia, yellow fever, epidemic jaundice, icterus gravis, etc.—that this variety of jaundice is generally met with.



Pylorus.

*Opening of
Bile ducts*

70 .vml
ABXORUAO

PROTEOSES IN SEROUS EFFUSIONS.

By W. D. HALLIBURTON, M.D., F.R.S., *Professor of Physiology*; and P. C. COLLS, *Junior Demonstrator of Physiology, King's College, London.*

From the Physiological Laboratory, King's College, London.

SOME years ago one of us¹ examined a large number of various drop-sical effusions, and arrived at the conclusion that these fluids contained no proteoses (albumoses) or peptone. To this rule cerebro-spinal fluid was the only exception. We have been led to take up this subject again for two reasons. The first of these is, that since the papers referred to were written, improved methods have been introduced for the detection of the substances in question; and the second is, the appearance of a paper by Dr. A. Lockhart Gillespie² on the same subject. Dr. Gillespie examined twenty-two cases of various effusions (pleural, ascitic, cedema, amniotic), and found proteoses in all cases, and true peptone in seventeen out of the twenty-two. In some cases traces only were found, but in the majority sufficient was present to enable him to estimate the quantity, and to separate the proteoses into their varieties. He further states that while he does not know their significance, the presence of these substances must be due to the action of some of the digestive ferments which are known to be present normally in the blood. The only proteolytic ferment which has been found with certainty in the blood is pepsin; and as this requires an acid medium for its action, it is difficult to understand this explanation. However, before seeking an explanation we considered it best to make sure of the facts, and, as the sequel will show, our conclusion is that proteoses and peptones are always absent from serous effusions, and that Dr. Gillespie's work is vitiated by the use of faulty methods.

We will therefore first consider the question of methods, and pass subsequently to our results. The principal methods for the detection of proteoses and peptone in fluids which contain also albumins and globulins, are four in number—

¹ W. D. Halliburton, *Brit. Med. Journ.*, July 26, 1890; *Journ. Physiol.*, London, vol. x. p. 232. "Text-Book of Chemical Physiology and Pathology," chap. xviii.

² *Rep. Lab. Roy. Coll. Phys.*, Edin. 1894, vol. v. p. 51.

1. Heat coagulation method.
2. Devoto's modification of the ammonium sulphate method.
3. The alcohol method.
4. The trichloroacetic acid method.

These we will take one by one.

1. *Heat coagulation method.*—This method consists in heating the mixture, acidifying slightly with acetic acid, filtering off the coagulated albumins and globulins, and examining the filtrate for proteoses and peptone. This was the method used by Gillespie. He says: "The albumoses were obtained by saturation of the specimen with ammonium sulphate after removal of all the proteids coagulable by heat, and separated into their three forms by means of sodium chloride and dialysis." This method is now practically entirely abandoned by those who wish to obtain trustworthy results. It has been found that heating such an acid mixture leads to the actual formation of proteoses and peptone from the native proteids; it is therefore perfectly easy to understand that he succeeded in finding these products of hydration in the effusions he examined.

The use of this method has led to many similar mistakes in the past: it led Hofmeister to describe proteoses and peptone as a constituent of the blood; it led Struve, Schmidt-Mulheim, and others to the conclusion that a peptone-like substance exists in milk; and more recently Chabrié,¹ by the use of the same method, has described a new constituent of blood serum, which has the properties of a proteose, and to which he has given the name albumone. Chabrié's mistake has been amply demonstrated by Robert Brunner.²

Neumeister, Sidney Martin, Starling, Halliburton, and others, in numerous papers, have also protested against the use of this method.

2. *Devoto's method.*—This method consists in saturating the proteid mixture with ammonium sulphate, boiling and filtering. The filtrate will contain the peptone, if any is present. The precipitate on the filter is then extracted with boiling water. The extract will contain the proteoses, if any are present. This method is a decided improvement on the one first described, as it avoids the use of acid; we have, however, found it is not wholly free from the same objection as was urged against the first method, for the boiling alone will cause the formation of a small amount of primary proteose; thus a fluid which, by absolutely trustworthy tests, is shown to contain no proteose or peptone, will often give a slight indication of proteose by Devoto's method.

M. Matthes³ has arrived at the same conclusion. His special object was to search for proteoses and peptone in the blood of leucæmic patients; his preliminary experiments led him to abandon Devoto's method in favour of the alcohol method, to be immediately described.

¹ *Compt. rend. Acad. d. Sc., Paris*, vol. cxiii. p. 557.

² "Inaug. Diss.," Berne, 1894.

³ *Berl. klin. Wchnschr.*, bd. xxxi. s. 351.

Wright¹ and Pekelharing² have considered that the non-coagulability of dog's blood, often noted after injection of nucleo-albumin, may be due to the splitting of the material injected into a nuclein moiety and a peptone moiety, and that the latter is responsible for the non-coagulability of the blood. This was supported by Pekelharing's finding peptone in such blood. C. J. Martin³ and Halliburton and Brodie⁴ have been unable to accept this explanation, chiefly because they have not been able to confirm Pekelharing's observation. For the detection of peptone (the term as here used includes the proteoses) Pekelharing used Devoto's method, which, as we have just seen, is untrustworthy.

3. *The alcohol method.*—This method consists in coagulating albumins and globulins by the prolonged action of large quantities of alcohol. After some months, water dissolves out from the precipitate so produced, proteoses and peptone, the albumin, globulin, and other native proteids being entirely insoluble. This is the method which Sidney Martin⁵ has employed with such fruitful results in his investigations into the proteoses of disease (diphtheria, tetanus, etc.) which accumulate in the spleen and elsewhere. It is the method by which Gourlay⁶ investigated normal spleens, and so corrected v. Jaksch's⁷ statement that normal spleens contain "peptone." It is a method which is especially adapted to the investigation of the proteids obtainable from solid organs; but it also works perfectly well with liquids—indeed the statement originally made by one of us, already referred to, that serous fluids are free from proteoses and peptone, and that cerebro-spinal fluid contains such substances, was the result of experiments principally carried out by the use of this method. It is absolutely trustworthy; the only disadvantage it possesses is the length of time it takes.

4. *The trichloroacetic acid method.*—This method is also perfectly trustworthy, and possesses the additional advantage of being rapidly applied. It has been employed by Obermayer,⁸ Starling,⁹ C. J. Martin,¹⁰ and Halliburton and Brodie.¹¹

The method consists in adding to the suspected liquid an equal volume of a 10 per cent. solution of trichloroacetic acid. An abundant precipitate thus produced is filtered off, and the filtrate tested for proteoses and peptone. C. J. Martin, however, pointed out that in the cold the proteoses would be partly in the precipitate. He therefore

¹ *Proc. Roy. Irish Acad.*, 3rd series, ii. No. 2, 1892, p. 117.

² *Verhandl. de k. Akad. v. Wetensch., te Amsterdam, Tweede Sectie, Deel i. No. 3.*

³ *Journ. Physiol.*, Cambridge, 1893, vol. xv. p. 375.

⁴ *Ibid.*, 1894, vol. xvii. p. 158.

⁵ Goulstonian Lectures, *Brit. Med. Journ.*, London, March, 1892.

⁶ *Journ. Physiol.*, London, 1894, vol. xvi. p. 32.

⁷ *Ztschr. f. physiol. Chem.*, Strasburg, 1892, bd. xvi. s. 243.

⁸ *Wien. med. Jahrb.*, 1888, ss. 375-381.

⁹ *Journ. Physiol.*, Cambridge, vol. xiv. p. 131.

¹⁰ *Ibid.*, vol. xv. p. 375.

¹¹ *Ibid.*, vol. xvii. p. 169.

recommends that after the acid is added the mixture should be rapidly boiled and filtered while hot. The filtrate, on cooling, deposits some of the previously dissolved proteoses, if any are present.

Starling advises that heat should not be applied for fear of the formation of primary proteoses. But C. J. Martin found, and we have confirmed his observation, that with rapid boiling and filtration this does not occur. The strong acid appears to have such a coagulating effect on the native proteids that the hydrating tendency of the hot acid has no effect on them.

This is the method, and it is an extremely delicate one, upon which we have relied in investigating the question whether proteoses or peptone are present in serous effusions.

More in detail, the actual *modus operandi* was as follows:—The serous fluid under investigation was divided into several portions, which were lettered *a*, *b*, *c*, etc.

a. This was saturated with ammonium sulphate in the cold, and filtered. The filtrate was tested for peptone by the biuret reaction.

b. This was saturated with ammonium sulphate, boiled and filtered. The filtrate was tested for peptone.

c. The precipitate on the filter in *b* was then extracted with boiling water, and filtered. The extract was tested for proteoses by the nitric acid test and the biuret reaction.

d. To this portion an equal volume of 10 per cent. solution of trichloroacetic acid was added, and filtration then performed. The filtrate was tested for proteoses and peptone.

e. This portion was treated in the same way as *d*, except that it was boiled before filtration.

f. This portion was treated with dilute acetic acid, boiled and filtered. The filtrate was tested for proteose and peptone.

The fluids investigated were the following:—Hydrocele fluid, six specimens; ascitic fluid, six specimens; pleural fluid, five specimens; fluid from cystic ovary, one specimen. For these we are chiefly indebted to Mr. Cyril Wace, House Physician, King's College Hospital; Dr. Willoughby Lyle, St. Peter's Hospital, supplied us with four of the specimens of hydrocele fluid, and Mr. P. T. Beale, with one. To all these gentlemen we beg to offer our sincere thanks for the assistance they have thus given to us.

The result of our investigations may be given under the same heads, *a*, *b*, *c*, etc., as the experiments just enumerated.

a. Peptone absent.

b. Peptone absent.

c. Traces of proteose occasionally found, but, as already explained, this method (Devoto's) is not free from error.

d. Proteose and peptone absent.

e. Proteose and peptone absent.

f. This experiment was not always performed, as we knew so well from previous work the fallacies of the method. In only one case did we fail to obtain evidence of proteose. It is no doubt possible to so adjust the amount

of acid, and the length of time during which the liquid is boiled, as to avoid the formation of proteose, but the chances are all the other way.

We may sum up our conclusions very briefly as follows :—

1. In searching for proteose or peptone in such fluids as blood, milk, or serous effusions, it is important to use such methods as will not in themselves lead to the formation of these hydration products from the native proteids present.

2. Of the methods described, boiling after acidulation, to coagulate the native proteids, is the one best calculated to lead to the formation of proteolytic products, and therefore the one least calculated to give trustworthy results.

3. Devoto's modification of the ammonium sulphate method is also not free from this source of error.

4. The methods which give good results are those in which either alcohol or trichloroacetic acid are used as agents for coagulating the native proteids.

5. The trichloroacetic acid method possesses the advantage of being rapidly performed; though in the investigation of solid organs like spleen, kidney, etc., the use of alcohol is preferable.

6. Our present experiments support previous conclusions, that serous effusions are free (like the blood from which they originate) from proteoses and peptone; and we consider that Dr. Gillespie's contrary conclusion is due to his having employed untrustworthy methods.

THE PHYSIOLOGY OF THE TRICHOPHYTONS.

(PRELIMINARY PAPER.)

By LESLIE ROBERTS, M.D., *Dermatologist to the Royal Infirmary,
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HITHERTO the study of the trichophytions has been pursued on botanical lines. Numerous publications in this and other countries have appeared within recent years, all dealing with the morphology of the fungi. But, strangely enough, the study of their physiology has been almost totally neglected. The first statement that had any bearing on this aspect of the question appeared in the writer's monograph on the Parasitic Fungi, printed for private circulation in 1893. I pointed out in a subsequent article¹ that the essential processes of ringworm of the scalp could occur in hairs detached from the scalp as dead bodies. I propose in the present article to give in detail a few experimental observations, which appear to me to throw some light on the physiology of the trichophytions. I shall endeavour to establish the fact that a certain group of lowly organised plants contain a *keratolytic* ferment, capable of digesting the keratine in horny animal tissues such as hair.

Since the brilliant researches of Claude Bernard we have become familiar with the fact that the processes of digestion are not exclusively the property of animals. The two types of digestion which this investigator named *exterior* and *interstitial* are both met with in the plant world, but among its lowly organised members, destitute of chlorophyll, such as the fungi, exterior digestion is one of the conditions of life. If we succeed in establishing this view of the trichophytic fungi it will afford us an infallible test whereby to determine whether a fungus is trichophytic or not. For, in this case, a trichophytic fungus will be a keratolytic one, more or less capable of digesting horny or keratine tissues. The test will be physiological, not anatomical, and least of all a cultural one. Moreover, it is a physiological test, which can be applied with ease and precision, as will be shown in the course of this article. Not least among its advantages is the fact that it does away with the *necessity* of inoculating living animals with the suspected fungus, inasmuch as the trichophytions can vegetate in dead hair.

¹ *Brit Med. Journ.*, London, Sept. 29, 1894.

The bearing of this proposition on the life of the trichophytions in the hair follicle is pertinent; it will enable us to understand how the fungus gains entrance to the hair, and the nature of the changes which follow its entrance. We shall be able to see how ringworm, or trichophytosis as we may call it, consists of a mingling of saprophytic processes with pathological reactionary cell processes, and to gain at once some insight into the nature of the disease and the cause of its chronicity.

I. EXPERIMENTAL PROOF OF THE KERATOLYTIC ACTION OF THE TRICHOPHYTONS AND OF THE KERATOLYTIC FUNGI.¹

The hairs to be acted on were taken from man (English and Italian [red and black]), the dog (Irish terrier), the horse (body hair and tail hair), the cow, the goat, the monkey, the coypu rat, the emu, the opossum, the hyæna, and the lion.

The specimens of fungi were various human scalp trichophytions, including a curious and hitherto undescribed Chinese variety, and favus (one specimen).

The *method of experimentation* consists of two parts—(1) the conversion of the trichophytic fungus into the vegetation form; (2) the feeding of this vegetation with the particular kind of hair to be tested. The apparatus which I employ for rearing the vegetations is very simple, and consists of six circular transparent glass dishes, $\frac{1}{2}$ in. in depth and 1 in. in breadth. The covers of these dishes consist of circular pieces of plate-glass, $\frac{1}{8}$ in. thick and rather more than $1\frac{1}{4}$ in. broad²; a rough-ground groove on the under surface receives the rim of the dish. For convenience sake these six covered dishes are fixed on a metal stage, 7 in. long and 6 in. broad. The means of fixation must be of such a kind as will stand the sterilisation temperature of 160° C. I find stout copper wire the cheapest. It can be readily twisted into a series of six circular loops, the starting and finishing points being secured by passing the wires through holes punched out of the thin metal plate. The whole apparatus must be protected from dust by a small bell jar or a flat dish, 2 in. deep and about 5 in. broad, and sterilised in the dry steriliser. Solid or liquid nutrient media may be used. An infusion of malt (10 per cent.), prepared as in the Pasteur Institute, answers admirably. This may be solidified with agar. A good soil is sterilised potato, but when this is used it is necessary to place a little thin pad

¹ The term "keratolysis" is already employed in dermatology in a loose and vague way to denote certain rare congenital conditions of the skin, in which exfoliation of the cuticle highly exceeds the normal degree. But the term is so admirably fitted to denote this fermentative action on hair, that I have ventured to reapply it in the new sense. The word keratolytic is parallel with proteolytic, which, perhaps, is a farther advantage in retaining it in this new sense.

² I have found it more convenient to have the covers $1\frac{1}{4}$ in. broad.

of spongiopiling on the bottoms of the shallow dishes¹ which, before inserting the potato discs, must be moistened with a few drops of sterilised water. The inoculation is effected by cutting off the intra-follicular part of the diseased hair with sterilised scissors and inserting it quickly in the soil. The apparatus is then placed in the incubator at 30° C.

In about 1 week the vegetation may be fed with the special hair to be tested. The hair itself is not to be sterilised, but all bacteriological precautions are to be observed in cutting off and introducing the fragments of hair.² The bell jar is now replaced and the apparatus put back into the incubator.

A number of human adult hairs were exposed for 22 days to the action of a specimen of favus. At the end of this time one of the hairs, which before the experiment had been accidentally damaged, was pervaded by the fungus; three others were invested externally by a sheath of mycelium, and the underlying cuticle was somewhat corroded. After nearly 3 months' exposure some of the hairs showed the presence of the fungus in their medullary canal, the substance of the hair or cortical plates being undamaged. Parallel experiment with a trichophyton showed that the interior of the hair was invaded as early as the end of the first week. A full comparison between favus and the so-called trichophytos is outside the aim of the present article, but the results obtained in this single comparative trial of their digestive powers appear, *prima facie*, to agree with our clinical observations of favus and ringworm; for the actual damage inflicted on the hair itself by the entrance of the favus fungus is considerably less than in ringworm. Further evidence is required on this point, for, as I shall presently show, the keratolytic action of a fungus may be negatived more or less by something in the hair itself.

In another experiment I exposed to the action of six specimens of the so-called trichophyton microsporon, hairs taken from a horse, an Irish terrier, a monkey, a coypu rat, a hyæna, and a lion. After a week's exposure they were examined microscopically. The *lion's* hair was surrounded by a beautiful sheath of fructifying mycelium, but no deterioration or digestion of the cortical substance had taken place that I could detect. The *hyæna's* hair was a deeply pigmented one, and at the end of the week was smothered under the growing vegetation. But no digestion of the hair had occurred and no trace of fungus could be detected in its interior. The cuticle was intact. The pigment which was so abundant in this hair had probably something to do with its greater resistance to the action of the fungus. To the question of the protective influence of pigment I must advert in another place.

¹ Of course the spongiopiling pads must be sterilised with the glass dishes.

² The fragment of hair must be at least shorter than the breadth of the surface of the vegetation, generally about $\frac{1}{8}$ in. in length is sufficient.

The *coyote rat's* hair had become overgrown with the *aspergillus fumigatus*, but the hair which was a pigmented one showed no signs of dissolving. The *monkey's hair* showed a slight external vegetation, but the walls were regular and showed no signs of weakness. *Irish terrier's hair*—At the end of 7 days there was an abundant external vegetation, rich in buds and ampullæ attached to the cuticle, but no internal vegetation. The contour of the hair was regular. On the ninth day there was a beautiful internal vegetation, confined, however, entirely to the medullary space. The segments of this internal mycelium measured from 6–7 μ , and were nearly spherical in shape.

The hairs taken from the *horse* showed after 7 days' exposure unmistakable signs of undergoing digestion. The cuticle was corroded and dissolved in parts and surrounded by a sheath of vegetation. The interior of the hair was almost filled with the fungus, and the cortical plates were converted into granular débris. The segments of the internal mycelium averaged 3–4 μ , those of the external growth about 40 μ .

These observations, few as they are, indicate the inequality of the digestive or keratolytic action of a given specimen of trichophyton in respect to different hairs. The more pigmented hairs resisted longer than the less pigmented ones. Those which were observed to undergo digestion within the first 9 days were derived from *domesticated* animals, the undigested hairs were those of animals living in a state of nature. We cannot, however, rightly infer that the hairs of wild animals have a complete immunity, for I shall presently show that the hairs of the opossum may be completely digested by trichophytic fungi. Nevertheless, broadly speaking, the practical immunity of wild animals from the attacks of these fungi is borne out by the experience of those who have the care of our Zoological Gardens.¹

A marked change of form in the mycelial cell takes place when the fungus gains the interior of the hair, and is in the stage of keratolytic activity. The cell in the condition of vegetation is long, the septa are comparatively far apart, the walls parallel. The shape of the digesting cell varies from the spherical to the cubical, one form being more commonly met with in one variety than in another; but in all varieties, without exception, the mycelial cell in a state of keratolytic activity is smaller than the mycelial cell in a state of vegetation.

None of the English, French, or German trichophytons that I have met with display the digesting faculty to the degree manifested by a curious *Chinese* specimen which I obtained from the head of a native in Tientsin, through the kindness of my brother, the late Dr. Frederick

¹ Mr. Thomson, the principal keeper of the Zoological Gardens, London, informs me in a letter, which he was good enough to send me in reply to my question concerning the immunity of wild animals, that ringworm has never been observed, to his knowledge, in any of the animals under his care. Dr. Harrison's testimony as pathologist to the Clifton Zoological Gardens agrees with that of Mr. Thomson.

Roberts of that city. The hairs, on their arrival, were transferred to a 10 per cent. infusion of malt, and I was fortunate in obtaining pure cultures of a curious description. Since this specimen has not hitherto been described I may mention some peculiarities which distinguished it. The original cultures, obtained directly from the imported hairs, grew very slowly in 10 per cent. infusion of malt at 30° C. Their growth energy was small, the colonies ceasing to enlarge after attaining the size of a pea. The individual colonies tended to fuse at their margins into a membranous vegetation. The aerial growths soon acquired a puce colour, but it was noted distinctly that some of the colonies did not form this colour, but remained colourless, though apparently in all other respects similar to the puce vegetation. The submerged growths always remained colourless. About 7 days after the development of the puce coloration white offshoots were observed. I found that the puce vegetations and the white offshoots could be cultivated separately, each maintaining their own characters. Cultivations of the white offshoots on sterilised potato discs, kept in a moist condition, developed as white fluffy vegetations of rather slow and limited growth. The puce form, when reared on agar beer wort (Sabouraud's formula), assumed the colour of magenta. Subsequent generations of the white form grew more rapidly, and microscopically showed the unmistakable fructification forms of the *aspergillus* group.¹ I may state briefly that the white forms, both early and subsequent generations, as well as the puce and magenta vegetations, were keratolytic. The following are my observations respecting the keratolytic action of these two specimens:—

On 24th October 1894 pieces of human hair were exposed to the action of the coloured Chinese vegetations. In 6 days there was some vegetation of fungus around the sheath, but the interior was sound and contained no fungus.

On 31st October several pieces of cow's hair and of dark red Italian human hair were exposed to the action of the white Chinese vegetation. In 7 days the hairs were examined. The colourless cow's hair was found more digested than the human pigmented hair. The fungus had entered both hairs by the cut ends, its filaments lay parallel to the long axis of the shaft, and were closely segmented. The portion of the hairs invaded were shrunken to one-half of the diameter of the unaffected portions.

On 13th November several pieces of goat's hair and Italian human hair were exposed to the action of the white and magenta vegetations. Thirteen days later I could not find a trace of the hairs laid on the magenta specimen. They had apparently been completely

¹ I will not venture to speak positively as to the species. The spherical ends of the gonidiophores had a yellowish-brown colour, the sterigmata were unbranched, the gonidia smooth colourless, and about 4–5 μ in size. It grows well at temperatures, varying from 25°–30° C. I find in my notes the name *Aspergillus flavus* (?)

digested. Some of the hairs exposed to the white specimen were examined in 7 days. The fungus had entered at the cut ends of the hairs, and so far as they had penetrated into the interior the cortical plates had been reduced to débris, but the cuticle remained undigested. After 16 days of exposure I could find only a few vestiges of the Italian hair. They were recognised amid the mycelial filaments by the pigmented cortical plates. The colourless goat's hair had disappeared completely, leaving no trace of its former existence. Here, again, I would point out that the presence of pigment in the hair rendered the process of digestion more difficult, as was well shown by the greater resistance which the dark pigmented Italian hairs offered to the fungus as compared with the colourless goat's hair.

On 1st December hairs from a horse's tail, an opossum, and a fragment of a hair-like feather from an emu were exposed to the action of the white Chinese vegetation of the third generation, grown on potato discs. The vegetation showed, microscopically, the fructifications of an aspergillus. Eleven days later, I examined the hairs and found the deeply pigmented tail-hair of the horse, and hair-like feather of the emu quite unaffected, but the hair of the marsupial was evidently undergoing digestion. The fungus was seen to have entered by the cut end, and to have undergone a considerable change of form, looking more like an ordinary trichophyton in the interior of a human hair.

On 12th December I determined to re-test the keratolytic action of the aspergillus fungus, and a fresh generation having been properly reared on sterilised potato discs, pieces of Italian human hair (auburn and black) were exposed to its action. When, 14 days later, some of the hairs were examined, they were found to be partially digested. After 49 days there remained only a microscopic vestige.

No doubt remains in my mind that this form of aspergillus is keratolytic. We have here an instance of two fungi differing morphologically as widely as an aspergillus from a trichophyton, and yet both physiologically referable to the same group. What, we may ask, is the genetic relationship between the magenta-coloured vegetations which appeared first, and the white offshoots which in *successive generations* developed later? I think we may dismiss the idea of its being an accidental impurity. Its uniform reappearance in successive generations is opposed to this supposition, while the physiological test which I applied to it demonstrated it to be keratolytic. This observation is not unique; Kral in Prague and Bodin in Paris have recorded instances in which certain cryptogams appeared regularly in successive generations of favus and trichophytic vegetations.¹ I proposed to speak of them generally as *physiologically associated fungi*, but any further discussion as to their mutual relationship would be premature in this stage of our knowledge.

How the work of digestion is carried out has yet to be demonstrated,

¹ Bodin, *Ann. de dermat. et de syph.*, Paris, 1894, tome v. p. 1239.

but I think there can be little doubt that it is affected by a digestive ferment.

According to Allan Macfadyen¹ certain trichophytic fungi, examined by him, produced a proteolytic enzyme, which liquefied gelatine very rapidly. Unfortunately, Dr. Macfadyen's paper is too brief and categorical for us to form a positive opinion on the subject.² A discussion on the exact nature of the process would be premature at this stage, when no thorough chemical investigation has yet been published. Reasoning from the nature of ferments in general, I believe it will be shown that the keratolytic ferment is not an enzyme but an organised ferment or zyme. The peculiar character of an enzyme, or soluble ferment, as defined by A. Meyer in his *Chemistry of Fermentation*, is that the quantity of the ferment "stands in inordinately small relation to the magnitude of the chemical processes caused by it." Now, in the digestive work of the trichophytons we note that the process of solution takes place only in the immediate vicinity of the fungus, and that parts of the hair not in actual contact with the organism remain intact. But if the ferment were an enzyme we should expect that by diffusion through the hair its keratolytic action would be brought to bear on remoter parts, which, as we have seen, is not the case. And, again, we have no evidence to-day that the digestive action can continue after the death of the fungus, or separate from its immediate life. In a word, the process is a physiological rather than a chemical one. It is quite possible that ferments of both kinds may coexist, as is actually the case with the yeast cell, which contains a soluble chemical ferment capable of converting cane sugar into invert sugar, a mixture of dextrose and lævulose, and an alcoholic ferment which, so far as we know, is inseparable from the life of the cell. The conditions under which this keratolytic ferment works are those which control the operations of all ferments, namely, the presence of water and a certain range of temperature. The experiments related above were conducted at a temperature varying from 25° to 30° C. The maximum growth-energy is attained at about 31° or 32° C. From 35° C. to 38° C. marked morphological changes occur, corresponding with certain obscure chemical changes, as manifested by the formation of pigment in previously colourless specimens. I have not yet tested the keratolytic energy at these higher temperatures, but on *à priori* grounds we may reasonably presume that its highest activity corresponds with the maximum growth-energy of the fungus.

We may reasonably ask ourselves whether any variations are discernible in the physiological working of the trichophytic fungi? To this question I can give but a guarded reply, since, in order to establish the fact

¹ *Brit. Med. Journ.*, Sept. 22, 1894.

² Since the above was written, the full text of Dr. Macfadyen's investigation has appeared in the April number of this journal. His investigations confirm in me the belief that the keratolytic ferment is distinct from the proteolytic.

of a true variation in any species of living organism, it is necessary to observe them through a very wide range and under diverse conditions. But so far as my observations have gone I am inclined to recognise two varieties of keratolytic action, namely, the kind which destroys cuticle and cortex simultaneously, and that which digests the cortex, leaving the cuticle of the hair, or dissolving it at a later period. The Chinese specimen belongs to the latter group. The diseased hairs from the head of the Chinese boy, when examined under the microscope, showed that the cuticle was intact, but the interior substance of the hair was reduced to a fine débris, which was floated out by a current of warm liquor potassæ, through the cut ends of the hair, leaving an empty shell of cuticle. In the observations on the keratolytic action of the Chinese vegetations the same tendency was noted, only eventually the cuticle itself was digested. This elective tendency may be noted clinically in patients affected with scalp ringworm. Thus, out of the last 40 consecutive cases of scalp ringworm seen in my private and hospital practice, in 22 both cuticle and cortex were destroyed, and in 18 the cortex only.¹ The constancy of this feature, considered moreover in the light of certain peculiarities in their clinical history, has led me to regard it as a natural variation.

Our attention was first drawn to these two varieties by the thorough investigations² of Sabouraud. His conclusions, however, are in my opinion not sanctioned by reason or experience. They are too positive and sweeping in their nature. Thus he differentiates *Tinea tonsurans* "into two separate classes, as distinct one from another as *tinea tonsurans* is, for example, from *favus*." This distinction he bases "on the different modes of development of the organs of fructification." He therefore, arrives at the conclusions on morphological lines, while I draw my inferences respecting true variations in the trichophytic fungi entirely from the study of their physiological habits. Our standpoints being so different it is quite obvious that we cannot concur in our conclusions. Thus, according to Sabouraud's view of the matter, *tinea tonsurans* is a definite disease of two types produced by distinct classes of fungi.

These classes are—(1) *tinea* with small spores due to the *Microsporon andouini*; and (2) *tinea* with large spores, trichophytic *tinea*, which is due to diverse forms of *Trichophyton megalosporon*. The latter class he again subdivides into two groups, according as the fungus vegetates outside the cuticle (*T. megalosporon ectothrix*), or within the substance of the hair (*T. m. endothrix*). Here we have a very convenient "cut-and-dried" system, and withal very arbitrary. But are we

¹ The proportion between one kind and another varies somewhat in every practice, so that no fixed percentage can be given. Generally the ratio is not so equal as that given in the text, the proportion of the cuticle and cortical destroying fungi to the others being roughly as two to one.

² *Ann. de dermat. et de syph.*, Paris, 1892, tome iii. p. 1061; *ibid.*, 1893, tome iv. p. 116; *ibid.*, 1893, tome iv. p. 814; *ibid.*, 1893, tome iv. p. 561; *ibid.*, 1894, tome v. p. 37; *Ann. de l'Inst. Pasteur*, Paris, 1894, vol. viii. p. 83.

to fit the facts to the "systems," or the systems to the facts? The latter is generally considered more scientific, and if we apply this recognised procedure to Sabouraud's pigeon-hole system, we find that the facts do not always fit in. For example, he arbitrarily separates the cuticle and cortical destroying variety from the true trichophytons, under which he includes the cortical destroying fungi, and which he has entitled the "trichophytic tineæ." Now I have proved, to my own satisfaction at least, that the essential bond between all trichophytic fungi is their digestive or keratolytic function. On what grounds, therefore, does Sabouraud separate the cuticle and cortical destroying fungus from the other members of the same group? He tells us, "by the botanical study of the development of the organs of fructification." Those who are thoroughly conversant with the botanical features of the whole group of the *Fungi imperfecti* (gymnomycetes, hyphomycetes), are well aware that the organs of fructification in their complete forms and in regular functional activity are never met with; and in the more lowly organised, such as the trichophytons, not a vestige of the higher homologues of the series is to be discovered. The so-called "organs of fructification" are merely vicarious forms of reproduction, consisting in the formation of buds; and even this occurs only under exceptional circumstances. How, then, is it possible to establish any dogmatic classification on the form of a structure in the highest degree variable and inconstant? Sabouraud's attempt to do so has led him to a *reductio ad absurdum*.

Want of space will only permit me to select two more examples to illustrate the perniciousness of this pigeon-hole system. The cortical-destroying fungi or "large spore tineæ," Sabouraud subdivides according as he finds them vegetating outside the cuticle or within the hair substance. Some time ago I reported observations which showed that the "endothrix" fungus may, under different circumstances, become an "ectothrix" vegetation. In a recent number of the *Giornale italiano delle mal. vener. e della pelle* (Sept. 1894), Prof. Mibelli has recorded certain facts which, unfortunately for the system, refuse to fit into Sabouraud's pigeon-holes.¹ Here are the facts. An Italian peasant keeps a cow which is affected with tineæ; the disease passes into the family, attacking the male servant, a child, a son, and the father. There is no doubt that the fungus passed from the animal to the various members of the family. In the child the hair follicles in the eyelid were affected, and here the fungus was *Trichophyton endothrix*, there being no external vegetation. In the father the disease took the form of sycosis barbæ, attacking the hair-follicles of the chin, and here the fungus was *Trichophyton ectothrix*, on account of the luxuriant mycelium which surrounded the cuticle outside the hair. There is no reason to doubt that the fungus was of the same kind in both positions. In the chin, follicles were favourable for the growth of an external vegetation,

¹ An English abstract in *Brit. Journ. Dermat.*, London, 1895, vol. vii. p. 64.

while the eyelid follicles were unfavourable; in this way we may roughly account for the morphological variation.

A still more forcible example is given by the same observer. An officer, aged 26, had patches of ringworm on the eyelid, face, neck, and beard. In the cilia the fungus was confined entirely to the interior of the hair, while in the chin follicles there was a luxuriant mycelium around the cuticle of the hair. No more pertinent example could be chosen to show the weakness of this system.

The facts I have recorded in the preceding pages seem to indicate the following conclusion. That there exists in the lowest orders of plants, destitute of chlorophyll, an extensive and natural group of fungi whose distinguishing feature is their ability to *digest* horny tissues, probably by means of a ferment; that this *keratolytic group* includes favus (achorion), the various kinds of trichophytions, and some aspergilli, and probably many others not yet identified; that there are at least two natural distinctions observable in the purely trichophytic fungi, namely, a kind that digests both the cuticle and cortical substance of the hair simultaneously, and a variety that digests the cortical substance, first leaving the cuticle unaffected, or attacking it at a later period.

A good deal of paraphrase may be avoided by introducing two new words to indicate these varieties. Sabouraud's terms, "microsporon" and "megalosporon" are so deeply involved with theories and misconceptions that we should do well to avoid them altogether. May we not designate the cuticle and cortex destroying fungi as *Trichophyta deformans*, since the contour of the hair, as seen under the microscope, is utterly deformed; and the cortex destroying fungi as *Trichophyta vagans*, on account of their wandering proclivities and tendency to attack individual hairs and small groups of hairs. If this suggestion were adopted, much misconception and errors in theory might be avoided in the future by those whose duty will be to study the pathology of the parasitic diseases of man.

THE MYCOLOGICAL PROCESSES OF THE INTESTINES.

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A MICROSCOPICAL examination of a minute amount of faecal matter gives the observer an idea that the number of different species of bacteria in the faeces is enormous. By further investigation, *e.g.* by the methods of isolation in common use, this somewhat exaggerated idea is no doubt corrected to a certain extent, but the fact remains that the different species capable of isolation from the intestinal contents, especially of the lower bowel, *may* be considerable. That this is the case is by no means remarkable, since it is obvious that not only *must* a large number of different microbes enter the alimentary canal with the food and drink under ordinary circumstances, some of which pass through the stomach without injury, but that, as a matter of fact, there is nothing to prevent any kind of organism, if sufficiently prevalent, from entering the stomach and from taking its chance of passing through that organ. The anti-septic power of the gastric juice has been abundantly demonstrated, and no doubt it is exercised in destroying large proportions of the bacteria which come within its sphere of influence, but there are circumstances which limit somewhat the destruction of micro-organisms by this germicidal action. Of these circumstances may be mentioned the following:—Introduction of the bacteria at the very end of a meal, when the stomachic contents are being passed on into the duodenum; introduction when the gastric mucous membrane is not in an active condition or in a vehicle such as water, which, as has been pointed out,¹ does not call out the maximum activity of the gastric glands; introduction when the gastric juice is abnormally weak from imperfection of function; and, finally, introduction of the bacteria in the condition of spores against which the active gastric secretion does not appear to be potent.

¹ Macfadyen, "Behaviour of Bacteria in the Digestive Tract," *Journ. Anat. and Physiol.*, London, Jan. and April, 1887, has shown that anthrax bacilli free from spores, if given in water to dogs after a fast, will pass through the stomach unscathed, and may be recovered from the intestines. Observations by others confirm and extend this.

Under any of these conditions, then, it appears to be possible for bacteria, particularly when introduced in large amount, to pass through the stomach unscathed in sufficient numbers to be capable of increase in the intestinal secretion. There are no doubt other such conditions.

There is reason to believe, however, that different species of bacteria have different powers of resisting the action of the gastric juice, some being much more able to withstand the full power of the secretion than others,¹ but this does not affect the main thesis, that conditions may arise under which any bacterium may pass through the stomach unscathed, and so appear in the intestinal contents. Such being the case, we find that if a list is drawn up of micro-organisms, which have been isolated by different observers from the intestinal contents, it will be found to contain at least fifty to sixty different species, independent of those species which have been, for the sake of experiment, passed through the stomachs of different animals,² for the express purpose of testing this question.

When we carefully consider one by one the different species in such a list, as enumerated in a compendium of bacteriology,³ we are able to distinguish two main divisions into which it would be easy to classify the majority of species, namely—(a) bacteria of the intestine, those species whose usual habitat may be considered to be the bowel; and (b) bacteria in the intestine, or of the intestinal contents, namely, those species whose presence in the intestine must be considered to be more

¹ In some comparative experiments upon this subject reported to the Local Government Board, 1889 to 1890, for example, I showed that the *B. prodigiosus*, the *B. tuberculosis*, the bacillus of grouse disease, and the bacillus of pneumonia could be isolated from the intestinal contents after having been administered by the mouth to animals, but that, under similar circumstances, the bacillus of typhoid fever and the bacillus of cholera (comma bacillus) could not be isolated.

² See, for example, report and further report, on "Bacteria in their Observed Relation with the Digestive Processes," *Medical Officer's Report, Local Government Board, 1889 to 1890* (V. D. Harris).

³ For the sake of illustration it will not be uninteresting to enumerate the following list given by Sternberg, "Manual of Bacteriology," New York, 1892.

The following species have been isolated from feces, and the contents of the intestine of cadavers:—

Non-pathogenic.—*Streptococcus coli gracilis* (Escherich), *Micrococcus aerogenes* (Miller), *M. versatilis* (Sternberg), *M. ovalis* (Escherich), Yellow liquefying staphylococcus (Escherich), *Porzellanococcus* (Escherich), *Bacillus subtilis*, *B. aerogenes* (Miller), *Bacterium aerogenes* (Miller), *B. lactis erythrogenes* (Hueppe), *Clostridium fetidum*, *B. muscoides* (Liborius), *B. putrificus coli* (Bienstock), *B. subtilis similis*, 1 and 2 (Bienstock), *B. zopfii*, *B. liquefaciens communis* (Sternberg), *B. intestinus liquefaciens* (Sternberg), *B. intestinus motilis* (Sternberg), *B. fluorescens liquefaciens* (Flügge), Colourless fluorescent liquefying bacillus (Escherich), Yellow liquefying bacillus (Escherich), *B. mesentericus vulgaris*, Bacilli of Booker, a to t of first series; a to s, second series; Bacilli of Jeffries, a to z, and α and β.

Pathogenic.—*Staphylococcus pyogenes aureus*, *B. typhi abdominalis*, *B. septicæmiæ hæmorrhagicæ*, B. of Belfani and Pascarella, *B. enteritidis*, B. of Lesage, *B. pseudo-murisepticus* (Bienstock), *B. coli communis* (Escherich), *B. lactis aerogenes*, *B. clavicida* (Brieger), B. of Emmerich, *B. coprogenes fetidus* (Schottelius), B. of Utpadel, *B. leporis lethalis* (Sternberg), *B. acidifromans* (Sternberg), *B. cuniculicida havaniensis* (Sternberg), *Proteus vulgaris* (Hauser), *B. tuberculosis*, *Spirillum cholerae Asiaticæ*, *Spirillum* of Finkler and Prior.

or less accidental. Others may be considered to be on the border line between these two classes. Of course we are aware that such a distinction is more or less artificial, since there is no particular reason why one micro-organism more than another should be found in the intestine, except the fact that such is actually the case. My experience in all my investigations into the bacteria which may be included under the first head, leads me to the belief that they are comparatively few in number, and in this idea I have been confirmed by the experience of others who have worked in the same direction. To this question I shall return in the description of those bacteria which have been isolated.

OF THE CHEMICAL PROCESSES ATTRIBUTABLE TO INTESTINAL BACTERIA.

Ever since the time of Kühne's first pronouncement upon the subject, various chemical processes, more or less connected with the digestion of the food in the intestines, have been put down to the action of micro-organisms in the intestine, and as time advances and a greater appreciation of the powers of microbes to bring about important chemical action is being arrived at, a larger and larger number of such chemical actions are being ascribed to what are sometimes called by physiologists, in a wide generalisation, the "putrefactive changes" which go on in the alimentary canal. Of such actions the chief would be considered to be the following—(a) the conversion of starch into sugar; (b) the conversion of proteid into peptone; (c) the splitting up of fats; (d) the conversion of peptones into substances, such as leucin, tyrosin, or into indol, skatol, etc.; (e) the production of acids such as acetic? lactic, butyric, or propionic; (f) the inversion of sugar; and (g) the splitting up of cellulose. In addition to these actions the splitting up and the rendering inert of the poisonous substances, such as choline, of which there should be a distinct amount from the decomposition of lecithin, a process connected so largely with organic substances taken as food, etc., and also the destruction of any poisonous alkaloidal substance formed in the intestine itself. Many of these actions are more matters of inference than of actual proof.

OBJECT OF INVESTIGATION.

In the investigation into what may be considered the usual mycological processes, which take place in the intestine, we have naturally confined attention to the action of those organisms which are of most frequent occurrence in the intestines, those included in the class above mentioned, *i.e.* bacteria of the intestine, and our first object has been to obtain such organisms by the methods at our command, plate-cultivations, etc. Having isolated as many different species as possible from the intestine (chiefly for the sake of comparison) of different animals, we have selected those species which have appeared most constantly,

and in largest numbers, and have grown these in different media in which one or other kind of proximate principle has been in excess; the third stage in the research consisted in bringing together certain of the species obtained from different animals, comparing them morphologically and biologically, and finally by injection into animals to ascertain whether or not some differences do or do not justify description of such organisms under different names. In the present paper it is intended to describe briefly the chief kinds of bacteria which have been isolated, and to give an account of some of their actions under different circumstances. It is proposed to postpone to another paper certain other actions as well as the general comparison between the organisms most alike in their morphology and method of growth, etc.

I. THE MICRO-ORGANISMS ISOLATED.

Method of procedure.—The animals used for the purposes of experiment, in addition to the human subject, were the cat, the guinea-pig, the rabbit, and the white rat. In the case of these animals the plan of procedure was as follows:—The animals were subjected to a fast of about 24 to 36 hours, and were then killed under chloroform. The abdomen having been opened, with antiseptic precautions, a small hole was made into the intestine at the various situations in which it was intended to examine the mucous membrane, a scraping of the mucous membrane at each of these situations was made with a platinum wire, and each such scraping was well diffused throughout a half-test tube of sterilised water or normal saline solution, and plate-cultures were obtained from the diluted solution. In this way various species of organisms were capable of being isolated. In the case of the human subject similar cultivations were obtained from scrapings of the intestinal mucous membrane of the cadaver. On the plate-cultivations thus obtained it often appeared at first sight as if a very large number of different forms were present, but on more careful examination it was generally found that the many forms could be resolved into few absolutely distinct species. It should be said that although cultivations from all parts of the small intestines, and indeed of the large, were made, most attention was devoted to those from the duodenum and jejunum, for two reasons—first, that in those parts of the intestines the chief chemical actions connected with micro-organisms in all probability occur; and, secondly, from these parts it was easier to obtain specimens of the intestinal mucus itself free, after only a short fasting period, from accidental faecal material.

The question of anærobic bacteria.—It has been assumed that the bacteria of the intestinal mucus are anærobic in their method of growth, because the intestine does not contain more than a small amount of free oxygen (estimations of the gases of the intestines in the human subject upon a meat diet, give according to Ruge, the following figures:—CO₂, 8

to 13; H, 0·7 to 3; CH₄, 26 to 37; N, 45 to 64, and traces only of O and H₂S). It is possible that there are a certain number of "strict anærobic" bacteria in the intestine, which will refuse to grow altogether in the presence of a trace of oxygen, but it is almost certain that the majority of the anærobes of the intestines are not strict, but "facultative" anærobes. Of the list of the intestinal bacteria given on page 311 it is only suggested that three or four are strict anærobes, *B. muscoides*, *Clostridium fetidum*, and *B. anærobicus liquefaciens*. A comparatively common variety is a facultative anærobe, namely, *B. caricida*, to which we propose to return in our next paper. It is thus seen that the criticism which is possible when we assert that the varieties of bacteria of the intestine are not many, namely, that there are many species present which will not grow except in the absence of air, is weakened by the fact that on investigation of the subject, by competent observers, it has been found that few of the intestinal bacteria are strict anærobes.

From the human duodenum and upper jejunum, we have been able to isolate six species of micro-organisms, of which only four can be considered to be constant, as having been found more than once. They are the following:—

1. *Not liquefying gelatine*.—Very small stout bacilli, almost of micrococcus form, slight difference between length and breadth, growing on plates in thick opaque white, irregular masses. In strokes, growing rapidly at the temperature of 20° C. on gelatine, opaque white with heaped-up advancing edge. Strong opalescent or tarry reflex. Rapid formation of arborisations behind growth. In stabs, growing both in the depth and upon the surface, with the same almost characteristic opaque whiteness. On agar, rapidly growing as a thick white creamy growth, with raised advancing edge. The growth always moist-looking. On potato as a thick heaped-up white growth, growing quickly in even 24 hours. The bacillus does not exhibit motility.

In many ways this micro-organism resembles the *Bacillus coli communis*, but there are distinct points in its way of growth which appear to separate it from that organism. It seems unnecessary to give it a name, and so for our purposes it may be indicated by the No. 1.

2. *Not liquefying gelatine*.—Small stout bacilli, about 1 to 2 μ long and $\cdot 5 \mu$ broad. Ends more or less pointed. No independent motility, or only very slight. Growing quickly under almost any circumstances. On plates as a very thin superficial growth, generally with a slightly heaped-up darker centre, especially if the colony has started slightly in the substance of the gelatine. Commencing as a more or less rounded colony but soon becoming irregular, edge very slightly raised if at all. Slightly yellow. In streaks, growing in small colonies which coalesce, and then rapidly spread over the surface of the gelatine as a very superficial thin deposit, with irregular edges. In stabs, growing scantily in the depth, but very freely on surface of the medium. On agar, as a thin extensive growth, with sinuous, not raised, edge. Marked metallic lustre. On potato, growing less rapidly than (1) and more localised to the point of inoculation, a dull almost frosted look, and distinct yellow colour.

From these and other considerations it would appear that this micro-organism is the *B. coli communis* (a).

In all the cases examined it constituted the chief species of micro-organism

found, quite outnumbering as regards colonies, any other, and apparently invariably present whatever the part of the intestine examined.

3. *Not-liquefying gelatine*.—Small stout bacilli, with ends more rounded than 2, and broader, about 2 μ . long and .5 to .75 μ . broad. Growing upon the surface of a gelatine tube, or upon a plate as a thin, transversely-wrinkled even deposit, very transparent. Rapid growth. Growing in the depth, but better upon the surface in a stab-culture. A particularly characteristic growth upon potato, a heaped-up, wrinkled, honeycombed growth, spreading all over the surface. Much the same appearance upon agar, the creaking and crackling appearance being particularly well marked.

4. *Liquefying gelatine*.—Longish bacilli with edges tending to be round. Motile (? flagellated). Longer than either of the other species described. In streaks or stabs quickly liquefying the gelatine, and after a time producing complete solution, and leaving some white flocculent deposit. No marked dulling of the dissolved gelatine. On agar, growing rapidly and producing a semi-liquefaction along growth, and a deposit of a white nature at end of growth. Slightly raised and undulating edge. On potato, very superficial, very slightly raised growth, localised, not growing freely or extending much.

This species probably belonged to the *Proteus vulgaris* group of organisms.

In the cat the following different species were isolated:—

5. *Liquefying gelatine*.—Large, stout, more or less oval rods. Probably actively motile. Liquefying the culture medium very rapidly in a deep furrow along the stroke of the platinum, the liquefied gelatine being dulled and with a white deposit, but no scum upon the surface. In stabs, giving a very broad funnel with a copious flocculent white deposit. On agar, extensive, rapid, thick, whitish growth, with opaque, whitish-yellow projections at the edges. On potato, thin, moist, extensive, rapidly-growing edge; deposit, yellowish.

6. *Not liquefying gelatine*.—Large streptococci. On plates and in streaks upon gelatine growing as a very thin, markedly yellow growth, and quickly. In stabs, growing sparingly in small isolated colonies, which did not coalesce in the depth, but freely upon the surface. On agar, as a thin, even, whitish streak, not growing freely, edges slightly raised. On potato, very imperfectly and simply for some time round points of inoculation.

7. *Not liquefying gelatine*.—Not markedly motile. Growing in plates as opaque yellowish spots. Longer and larger bacilli, probably as much as once and a half the size of 2. Growing somewhat slowly and superficially. In stabs, growing sparingly in the depth, but freely on surface. On agar, as a very thick moist mass, with a drop which runs up the bottom of the test-tube. On potato, heaped-up, thick, yellow, dulled mass, not growing quickly, or extending much, growing edge thin and even. This species resembles in growth the *B. coli communis* (β).

In the guinea-pig several species were isolated, but the following, as occurring constantly in the plates, were those with which experiments were made.

8. *Not liquefying gelatine*.—Very large, short, stout bacilli. More or less motile. On plates growing much as 7. In streaks presenting an even and regular, not rapid growth. White, not dead white, with a faint yellow reflex. Superficial, very slightly raised, and irregular growing edge. Not growing well in the depth of the gelatine in stab-cultures, but spreading upon the stabbed surface and upon the top. Upon agar-agar, superficial, rapidly-growing, white culture. Upon potatoes, covering the surface gradually, as a thin, superficial, dull yellow, never moist layer; growing much more slowly, however, than the

B. neapolitanus (Emmerich), with which it was compared. Probably *B. coli communis* (γ).

9. *Not liquefying gelatine*.—Short, stout, large bacilli. Similar in nearly all respects to 8, but growing in a much thicker streak. Hardly growing at all in the depth of the gelatine. Differing from 8 in this, that it produced no curdling in milk even after 6 days.

10. *Not liquefying gelatine*.—Large, short, stout bacilli, with rounded ends. Growing very superficially upon plates, and spreading rapidly over considerable surfaces; centre pale, with slightly-raised dotted edge, which has a distinct yellowish reflex. In stabs, showing quickly and much more constantly than 8 or 9, the arborescence in the depth of the gelatine. Growth in the depth, however, slow. On agar-agar, spreading white growth, uniform and thin. Not growing so well on potato as 8 or 9. Not readily curdling milk. The growths of 8, 9, and 10 were very similar, with slight points of difference, but the behaviour of the organisms, with respect to the precipitation of milk, was very different; whereas 8 curdled or precipitated milk in 22 hours, the other organisms did not do so in 6 days. In many respects each of these organisms might be considered as the *B. coli communis*, only the first, namely, No. 8, corresponded with the typical organism in this respect.

11. Various species of sarcina, of which *Sarcina lutea* was the most constant.

12. *Liquefying gelatine*.—Small, stout bacilli. In streaks, liquefying gelatine quickly leaving a clean gutter and a cloudy liquid with heavy brownish-white deposit. In stabs as in streaks, with thick, viscid, cloudy liquid and white deposit. On agar, rapidly growing and spreading all over the surface, white, regular, slightly-raised edges. Very moist, and apparently liquefying the agar to a certain extent. On potato, with a dull, uniform, frosty-looking yellowish growth, with slight orange tint, somewhat moist and not thick.

13. *Liquefying gelatine*.—Short, stout bacilli, almost micrococci, ends tapering slightly. In streaks, liquefying gelatine slowly, along a clean-cut gutter. In stabs, the liquefaction forms a shallow cup, and the course of the puncture very slowly converted into a narrow channel. Surface of gelatine covered with whitish growth. On agar, superficial, widely-spreading moist growth, with curved edge. On potato, thick, yellowish, heaped-up but not widely distributed growth, of deep yellow colour. No curdling of milk in 6 days.

From the rat ten different species were isolated.

14. *Not liquefying gelatine*.—Short, stout bacilli, scarcely broader than long. Growing moderately slowly in streaks, white opaque, superficial, with minutely undulating edge and a tar-water reflex about edges. In stabs, very slightly growing in the depth, but freely on surface of gelatine. On agar, thick spreading, but slowly growing white and moist, colonies not quickly coalescing. On potato, growing very imperfectly as a moist superficial scum, in almost all respects resembling 10 in its growth (δ).

15. *Not liquefying gelatine*.—Very fine small bacilli. In growth almost exactly resembling 14, but producing a moister growth, as though the gelatine were slightly dissolved. Both 14 and 15 more or less motile.

16. *Not liquefying gelatine*.—Large streptococci. On plates, small, opaque white colonies, round and regular, not coalescing in streaks. On agar-agar, growing slowly as small circumscribed colonies. Milk white. Not curdling milk.

17. *Liquefying gelatine*.—Short rods. In streaks, rapid liquefaction with granular deposit, furrow deep and clear, no dulling of the liquefied gelatine. Deposit, yellowish white. Growing on agar as thin, white, smooth, rapidly-spreading deposit; imperfectly upon potato. Not curdling milk.

18. *Liquefying gelatine slowly*.—Similar organism to 17, but giving a

yellowish deposit, dulling of liquefied gelatine, and a distinct, yellowish scum. Curdling milk.

19. *Rapidly liquefying gelatine*.—Large micrococci, producing granular cloudiness and white deposit and no scum. In stabs, an egg-cup-like liquefaction. On agar, growing as a long, thin, white streak, with slightly raised edges, not growing luxuriantly.

In addition to the above, several distinct kinds of *sarcina*, yellow and white, were isolated, and pink yeast was very often present. It seems unnecessary to give a description of more than the above.

In the rabbit, in addition to the *Sarcina lutea* and some species similar to those described above, the chief organism isolated was always present, and in numbers altogether surpassing those of any other sort.

20. *Not liquefying gelatine*.—Large, stout bacilli, with no tendency towards coccus forms. Ends only slightly rounded. Growing in plates with a dark centre and a light periphery. Growing edge undulating. In streaks on gelatine growing rapidly light and thin, but colonies are perhaps not so distinct as in specimens of *Bacterium coli*, as a rule. More or less milkiness of gelatine in neighbourhood, but no distinct arborescences in depth. Growth has metallic lustre. On litmus (neutral) gelatine produces very distinct blueing of neighbourhood. In stabs, grows but little in depth, without gas bubbles. On agar, thick, solid, white growth, rapid in extent (e).

From the above description it will appear that, from the intestine of each of the animals experimented with, a species of micro-organism was isolated, corresponding closely to the usual descriptions of the *B. coli communis*. Indeed, it may also be said that several species in one (or more) of the animals had a close likeness to this microbe. Observers have been able to isolate organisms from the intestine, generally of the human cadaver, and from cases of peritonitis, etc., etc., which differ in method of growth, all of which they have called by the same name. A considerable confusion, therefore, exists as to what the *B. coli communis* really is, and as to what are the characteristic features which may be used to distinguish it from similar intestine microbes. According to my experience, nearly every one of all the tests which have been proposed to mark out the true from the pseudo-organism breaks down somewhere or another. The term is used in the most slipshod manner, particularly by those who have not gone carefully into the subject. At the same time, the minute differences of the growth of the different varieties of this species (if they be varieties and not distinct species?) under what may be looked upon as perfectly artificial conditions are not by any means satisfactory. With a view to discover whether the apparently similar organisms have any points of distinction, we have compared as many kinds as we have been able to come across, as to their morphology, methods of growth in different media, and, finally, by injection into rabbits and guinea-pigs. The results of this comparison will be given in the continuation of the present paper.

II. OF THE CHEMICAL ACTIONS OBSERVED.

(a) *The formation of indol.*—Since the time of a former investigation into the question of the production of indol, undertaken nearly 10 years ago in conjunction with Dr. Tooth,¹ the production of this substance has been used as a test to distinguish different species of bacteria, and even from this point of view must be considered of importance. Kitasato² has shown that the production of indol by bacteria from bouillon cultures is not by any means always the same; some bacteria produce it quickly, others after a time, and others not at all. As a test, the production of indol indicated by the rose-red or crimson colour, produced by the addition of sulphuric acid, with or without the addition of a small quantity of nitrous acid, or of a nitrite to a cultivation suspected of containing it, the time during which the bacteria act is of importance. Kitasato found that the indol reaction thus produced in 24 hours from a cultivation of the cholera bacilli (comma bacilli) was very strong, stronger than that similarly obtained in connection with, for example, Brieger's bacilli (*B. cavioides*) or with Emmerich's bacillus (*B. neapolitanus*), but that the typhus bacillus (typhoid) did not produce indol at all. This negative reaction he suggested as important for the purpose of distinguishing that species of micro-organism.³ That indol is produced by only certain bacteria of the intestines, and not at all by others, our former investigations had shown; in other words, we came to the conclusion that it was a specific production, and was not, as had been before supposed, always present when proteid matter was decomposed or putrefied. The production of indol as a test does not, however, distinguish cholera bacilli from the bacilli (e.g. *B. coli communis*) found in healthy animals (as in man) in the intestines. Thus, following the plan of Kitasato, but using a larger quantity of fluid (peptone bouillon), we find that our different species may be thus classified with respect to the formation of indol:—

Production of Indol in 20-24 hours.	Slow production after 1 or more days.	No production in 6 days.
	Nos.	Nos.
a. Weak, (2)	1	3
β. Strong, (7)	5	15
γ. Strong, (8)	9	16
	10	17
δ. Very strong, (20)	11	...
Also, Nos. 4	12	...
6	13	...
	δ. Weak, (14)	...
	18	...
	19	...

¹ *Journ. Physiol.*, Cambridge, vol. ix. 220.

² "Die negative indol-reaction der typhus bacillen im Gegensatz zu anderen ähnlichen bacillenarten," *Ztschr. f. Hyg.*, Leipzig, 1889, s. 515, *et seq.*

³ It should be noted, however, that the value of the indol reaction as a test is doubted by M. Chantemesse ("Traité de médecine," p. 734), and also by MM. Rodet and G. Roux (Communication à l'Académie de médecine, Oct. 20, 1891).

Thus we see, as regards the production of indol, out of the 20 species treated of only 6 produced indol in the time—namely, 24 hours—used in the above-mentioned test. In about one-half of the cases in which indol was slowly produced, the reaction observed was very feeble, almost *nil*. It is interesting in this connection to notice that out of the 6 cases, in which the production of indol was rapid and marked, 4 were species or varieties, differing in certain points, which we consider to belong to the *B. coli communis* class.

(b) *The formation of leucin and tyrosin.*—By the cultivation of no one of the organisms isolated which have been tried could leucin or tyrosin be obtained from the nutrient material. On the other hand, in an antiseptic pancreatic digestion of fibrin (2·5 per cent. carbolised digestive solution), on one occasion we have obtained an excellent crop of crystals of both leucin and tyrosin. This appears to justify those who have ascribed the formation of leucin and tyrosin in pancreatic digestion to the prolonged action of the pancreatic ferments, an idea upon which in a former paper we have ventured to throw doubt. It should be said that the only test available to us has been the examination of the residue after concentration, etc., under the microscope. The chemical tests for leucin and tyrosin are such as require more than a minute trace of the substances. Under such circumstances, it may be that our observations are insufficient to justify a generalisation, but we feel quite certain that the formation of such substances in any appreciable amount by micro-organisms, such as we have examined, is exceedingly rare. We are unable to find any detailed account of the mycotic formation of leucin and tyrosin in an original paper, although, as we have mentioned before, this chemical action is given as being one of the intestinal, so called putrefactive, changes.

(c) *The precipitation of the caseinogen of milk.*—The curdling of milk, using the term in its ordinary sense, which simply implies the precipitation of the caseinogen, is a very common action of the intestinal microbes. This is easily explained by the fact that a considerable number of them very speedily convert sugar, either lactose, glucose, or indeed saccharose, into lactic acid, as will be described in the next section. The actual formation of curd or caseine—*i.e.* coagulated caseinogen, is not common. Precipitation of milk took place very quickly in the case of the bacteria marked α , β , γ , δ , ϵ , the process was, as a rule, complete in 20 to 24 hours. It also took place in the other bacteria enumerated, with the exception of 4, 9, 10, 12, 13, 16, 17, 18, and 19. As the result of prolonged observation upon the time and nature of the curdling or precipitation of milk by different organisms, we came to the conclusion that, as a rule, the curdling was not a true coagulation of caseinogen, but that the precipitate could be redissolved in lime water and afterwards be formed into a true clot by the addition of rennet. To this subject, however, we hope to return in the following paper. In one case in which it was noted that there was never coagulation of milk, namely, with No. 19, the

caseinogen was converted into a soluble proteid, either a peptone or more likely an albumose. This was the most satisfactory example of the power which the bacteria showed of being able to convert proteids into albumose or peptone.

(d) *The conversion of starch into sugar.*—The diastasic power of bacteria is generally assumed to be common, even if not universal. It is true that some bacteria possess it to a high degree, as, for example, the *B. mesentericus vulgaris* (potato bacillus), but, according to our experience, very few of the intestinal bacteria do. The fact that so many of them show an abundant growth upon potatoes is no proof that they first of all convert the starch for their own growth into sugar; if such an action takes place at all it stops, one would believe, at dextrine. At any rate, bacteria grown in starch solutions of different strengths, to which $\frac{1}{2}$ to 1 per cent. of Liebig's extract has been added, do not, in the majority of cases, produce any reducing sugar. The starch is almost unchanged after several days' free growth, the solution being frequently acid from the formation (usually) of lactic acid. In only 2 cases was the presence of reducing sugar in *appreciable amount* demonstrated, namely, in 12 and 13.

(e) *The inversion of cane sugar.*—This action is generally believed to be of considerable degree in the intestines, and it is often thought to be mycological. It was carefully tested in the case of the chief bacteria above enumerated. A certain definite proportion of a solution of cane sugar was added to a $\frac{1}{2}$ per cent. solution of Liebig's extract, to an amount equal to 1 per cent. The reaction of the fluid was, if necessary, made neutral. The almost invariable action of the bacteria under such circumstances was to produce lactic acid in considerable amount, and in some cases other acids; the further formation of butyric acid was not noted, but probably occurred sometimes (to the formation of butyric and other acids such as proprionic acid we propose to recur in a future paper). With only one variety was the amount of reducing sugar present after a few days' incubation at 40° C., namely, with No. 4, a slight amount with Nos. 12 and 13.

(f) *The production of organic acids, particularly lactic.*—The production of lactic acid in solutions to which cane sugar was added, and in cultivation media, such as nutrient gelatine, to which a small amount of glucose was incorporated, was very common. Lactic acid was proved to be present in the liquid media in which was grown each of the five species of bacteria considered to be varieties of the *B. coli communis*. It was also present quickly in liquid cultivations of 9, 10, 15, 16, 17, and 19. It is a point worthy of considerable attention, namely, the speedy production of lactic acid in the stomach and in the intestines by bacterial action, supposing a considerable amount of sugar be present in food, and to a less amount if unconverted starch remain either undigested or unabsorbed; not one variety of bacterium but several distinct species seemingly having the power of producing this acid.

As regards other possible mycological actions in the intestines, particularly the formation of peptone and the splitting of fats, both of which are excessively rare, and certain other points connected with the differentiation of the chief species of bacteria which we have isolated, we propose to bring our observations together in a further paper.

I would desire to return thanks to the Laboratories' Committee of the Royal Colleges of Physicians (Lond.) and Surgeons (Eng.) for allowing me the privilege of working in the laboratories.

Note.—It should be said that the greatest care was taken in all the experiments to use the same amounts of each solution in which the bacteria were grown, and that the composition of the said solutions was, as far as possible, constant. When variations were noticed then in the growth of the different organisms, this source of fallacy was practically excluded.

Louis Pasteur.

BORN AT DÔLE, 27TH DECEMBER 1822—DIED AT GARCHÈS, NEAR PARIS,
29TH DECEMBER 1895.

THERE has passed from amongst us one of the greatest men of the century, not before his time, but great in work accomplished, having won the esteem of all men and that honour which is only awarded to men of noble purpose and high achievement.

Louis Pasteur was born at Dôle in the Jura, the ancient capital of Franche-Comté and originally the seat of the University College now at Besançon in the Doubs. What manner of people his father and mother were is best judged from the affection and esteem in which their distinguished son held them. Pasteur's education, commenced by his father, was continued at the Communal College of Arbois, at the University College of Besançon, and then at the Ecole Normale and the Sorbonne, where his first great investigation on the crystallography of the tartrates and the paratartrates was begun when he was still only about twenty-four years of age, and before he received his D.Sc. in 1847. This investigation was continued during his occupancy of the Professorial Chair of Physical Science in the Lycée at Dijon, whilst he was acting as Assistant Professor of Chemistry in the University of Strasburg, and after 1854 during the time that he was Dean of the Faculty of Sciences at Lille. This purely physical investigation was the real foundation of Pasteur's contributions to the solution of some of the great biological problems that were at that time occupying the minds of the scientific world.

After his experiments on fermentation of the tartrates and on the relation of specific micro-organisms to this and other fermentative processes, followed his classification of organisms, according to their form, and also according to the conditions of food, moisture, air, and temperature under which they could exist and carry on their special functions. Even at this early period he divided micro-organisms into *aerobies* and *anaerobies*, and pointed out that it is to the faculty of wresting oxygen from sugar that certain organisms owe their power of breaking down this substance into simpler molecules, during the rearrangement of which comparatively large quantities of alcohol

may be formed. Then came his experiments on the *Mycoderma aceti*, during which he found that the most complex bodies could be broken down, through the agency of a series of putrefactive and fermentative organisms, into the simplest products, the anærobic organisms doing one kind of work, the aërobic another. Through their agency all organic matter, as soon as it dies, is broken down into substances which may be assimilated by plants, the living in this way being enabled to subsist on the dead material that had been stored up by previous generations.

For his work on spontaneous generation, Pasteur was awarded the prize of the Academy of Sciences. Lister, in this country, taking Pasteur's observations as a basis, built up his antiseptic system, which, in his hands and in those of his colleagues (amongst them the late Thomas Keith, who was great both as a man and as a surgeon), revolutionised surgery, and converted it from a mere handicraft, though a great one, to a noble art and a living science. Lister, writing to Pasteur in 1874, says:—

“It gives one pleasure to think that you will read with some interest what I have written about an organism which you were the first to study in your memoir on lactic fermentation. I do not know whether you read the *British Medical Journal*, if so you will from time to time have seen accounts of the antiseptic system, which for the last nine years I have been trying to bring to perfection. Allow me, to take this opportunity of sending to you my most cordial thanks for having, by your brilliant researches, demonstrated to me the truth of the germ theory of putrefaction, thus giving me the only principle which could lead to a happy end—the antiseptic system.”

And it was indeed a happy end. The combined genius of these two men cleansed surgery from a great blot; first wounds and then wards were purified and maintained aseptic, and results hitherto undreamed of were obtained in erysipelas- and gangrene-infected wards, although better results still were obtained as the principles of treatment and ward disinfection became more widely recognised.

It is unnecessary to do more than mention Pasteur's further researches on fermentation and putrefaction. Of his investigations into the cause and prevention of *pebrine* and *flacherie* in the silk-worm, diseases which at one time threatened to destroy the silk industry of France, much might be written, as the former was the first disease that was proved by carefully-planned experiments to be due to living parasitic (Cornalia's) corpuscles, the latter to microbes.

Robert Boyle prophesied that “he that understands the nature of ferments and fermentation shall probably be much better able than he that ignores them, to give a fair account of divers phenomena of certain diseases (as well fevers as others) which will perhaps be never properly understood without an insight into the doctrine of fermentations.” This prophecy was fulfilled in Pasteur's work, which, commencing with anthrax and ending with hydrophobia, was one long series of marvellous achievements, based on accurate experiments,

careful analysis, and brilliant deduction. The conditions necessary for the development of the full activity of the anthrax bacillus, the effect of temperature in lowering its vitality, were first worked out; then came the observations on the attenuation of the chicken cholera virus, by keeping it for a long time in the presence of air, and the successful protective vaccination experiments with this attenuated virus. This was followed by the production of a protective anthrax virus, a discovery to which medicine already owes so great a debt, and which promises still greater benefits to suffering humanity. In swine fever and in rabies Pasteur worked out the question of the exaltation and attenuation of a virus, by passing it through animals of different species. Of the practical outcome of his work on rabies, there remains little that has not been already often told and told well.

Though this investigation brought Pasteur's active experimental work to a close, he still remained in constant attendance at the laboratories in the Institut Pasteur in the Rue Dutôt, or in those connected with the stables at Garches, situated in the park of the old Château de Ville Neuve l'Etang, where the work on hydrophobia and diphtheria is carried on, guiding, advising, directing, and watching with keen interest the work of his pupils, encouraging them to overcome difficulties, and rejoicing with them in those great successes which have done so much to enable France to play so prominent a part in the advancement of medical science.

Pasteur was, as lovingly remarked by his friend and disciple Duclaux, a master amongst masters, a teacher amongst teachers, but he was something more—he was a great man. When, on the occasion of the celebration of his seventieth birthday, his admirers and disciples met in the Sorbonne to do him honour, Pasteur struck a note which vibrated in the heart of everyone present.

“Je crois invinciblement que la science et la paix triompheront de l'ignorance et de la guerre; que les peuples s'entendront non pour détruire mais pour édifier, et que l'avenir appartiendra à ceux qui auront le plus fait pour l'humanité souffrante. Jeunes gens, jeunes gens, confiez-vous à ces méthodes sûres, puissantes, dont nous ne connaissons encore que les premiers secrets. Et tous, quelle que soit votre carrière, ne vous laissez pas atteindre par le scepticisme dénigrant et stérile, ne vous laissez pas décourager par les tristesses de certaines heures qui passent sur une nation. Vivez dans la paix sereine des laboratoires et des bibliothèques. Dites-vous d'abord: Qu'ai-je fait pour mon instruction? Puis à mesure que vous avancerez: Qu'ai-je fait pour mon pays? jusqu'au moment où vous aurez peut-être cet immense bonheur de penser que vous avez contribué en quelque chose au progrès et au bien de l'humanité. Mais, que les efforts soient plus ou moins favorisés par la vie, il faut, quand on approche du grand but, être en droit de se dire: 'J'ai fait ce que j'ai pu.'”

These pregnant words lay bare the soul and motive of the man more fully than could any long description and analysis of his work.

Those who at any time came in contact with Pasteur could not but be struck by his energy, directness, and simplicity; his industry,

pertinacity, and clear-sightedness; his wonderful powers both of experiment and of deduction, and most of all by that power of induction which, amongst scientific men, is found only in those who may lay claim to genius.

Whatever may be Pasteur's claim to honour, and how great that is, all now recognise, it undoubtedly rests upon the work which he has done in connection with the etiology and prophylaxis of some of the specific infective diseases. One line of thought, work, and reasoning is followed throughout. Each step is carefully probed and tested before a further advance is made, and once gained is never left until it has been made thoroughly sound as a point from which the next step may be made. This it is that makes Pasteur's work so reliable, and assures for it that immortality to which only truth can attain.

When Sir Joseph Lister, presenting the gold medal on the occasion above referred to, spoke to the following effect, he well voiced the opinions of those present:—

“Vraiment, il n'existe pas dans le monde entier aucun individuel auquel doivent plus qu'à vous les sciences médicales. Vos recherches sur les fermentations ont jeté un rayon puissant qui a illuminé les ténèbres funestes de la chirurgie et a changé le traitement des plaies d'un affaire d'empirisme incertain et trop souvent désastreux en un art scientifique sûrement bienfaisant. Grâce à vous, la chirurgie a subi une révolution complète qui l'a dépouillée de ses erreurs et a élargi presque sans limite son pouvoir efficace. La médecine ne doit pas moins que la chirurgie, à vos études profondes et philosophiques. Vous avez levé le voile qui avait couvert pendant des siècles les maladies infectieuses. Vous avez démontré leur nature microbienne; grâce à votre initiative, et dans beaucoup de ces à vos propres travaux spéciaux, il y a déjà une foule de ces desordres pernicieux dont nous connaissons complètement les causes. Cette connaissance a déjà perfectionné d'une façon surprenante le diagnostic de ces fléaux du genre humain et a indiqué la route qu'il faut suivre pour leur traitement prophylactique et curatif. L'originalité de vos travaux sur le rage était si frappante que, à part quelques ignorants, tout le monde reconnaît maintenant la grandeur de ce que vous avez achevé contre cette maladie terrible. Vous avez fourni un diagnostic qui dissipé à coup sûr les angoisses d'incertitudes qui haïssaient autrefois celui qui avait été mordu par un chien soupçonné de la rage. Rien que cela aurait suffi pour vous assurer la gratitude éternelle de l'humanité. Mais par notre système merveilleux d'inoculations antirabiques vous avez su poursuivre le poison après son entrée dans le système et l'y vaincre.” And he concluded, “Medicine and surgery are eager, on this great occasion, to offer you the profound homage of their admiration and their gratitude.”

At the tercentenary celebration of the anniversary of the foundation of the University of Edinburgh, Pasteur said that when a great man died his loss was mourned by all nations alike. This is, indeed, true of the man who said it, for although his loss is mourned by France, who has lost a truly loyal son, the loss mourned by the scientific world is infinitely greater, though the work that Pasteur did will never perish, and his pupils, friends, and assistants have already given proof that we may look to them, and not in vain, for the continuation of the noble work their master has commenced.

G. S. W.

CONTRIBUTION TO THE STUDY OF THE SERUM THERAPEUTICS OF DIPHTHERIA: AN EXPERI- MENTAL INQUIRY INTO THE RELATIVE MERITS OF SEVERAL METHODS OF IMMUNISING HORSES WITH THE OBJECT OF OBTAINING FROM THEM A REMEDY FOR THIS DISEASE.

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Two methods of immunising animals against diphtheria, for the purpose of obtaining from them curative serum, have hitherto been employed, namely, the injection of living cultures, and the injection of their products. The former method was thought to be inferior to the latter, until Dr. Klein¹ produced some very powerful serum by its means. Acting on his suggestion, I commenced, last November, to immunise two horses, with the object of comparing the relative merits of the two methods.

PREPARATION OF TOXIN.

For the treatment of the first horse, and for the estimation of the antitoxic power of the serum obtained from time to time, toxins were prepared by Roux's method. Fernbach flasks containing 500 c.c. of 2 per cent. peptone beef-broth, which formed a thin layer at the flat bottom of the vessels, were sown from recent broth-cultures of Löffler's bacillus. These were then kept in the incubator at 37° C. for about three weeks, during the whole of which time a current of moist air was drawn over the fluid. Finally, the cultures were filtered through a Chamberland bougie, and to the filtrate .5 per cent. carbolic acid was added to preserve it.

DIMINUTION OF THE TOXIC POWER OF FILTRATES.

These filtrates were found to retain their toxic power fairly well, when kept in bottles almost completely filled, well corked, and

¹ *Brit. Med. Journ.*, London, December 1894.

removed from the influence of light. The importance of excluding, as far as possible, all air from the bottles, was made evident by the following experience. Of a certain filtrate, when first obtained, the minimal fatal quantity for guinea-pigs was .2 c.c. per kilog.¹ At the end of 3 months, during which it had been kept in a bottle almost completely filled up to the rubber cork, in a dark cupboard, at the ordinary temperature of the laboratory, its minimal fatal quantity had fallen to .3 per kilog. Half the contents of the bottle were then used, and from this time forward the remainder of the filtrate was kept under conditions identical with the first, except that the bottle was now half full of air. The diminution of toxic power, under these conditions, was more than twice as rapid as before, and at the end of another 2 months its minimal fatal dose was only .6 per kilog.

THE HORSES USED.

Of the horses used for these experiments, No. 1 was a young cart-horse between 3 and 4 years old, which weighed over 13 cwts.; No. 2 was a cob 9 years old, and 9 cwts. in weight. It will be shown a little later that these animals differed greatly in their susceptibility to the diphtheria poison. It is much to be regretted that animals more nearly resembling each other could not be obtained.

THE ANTITOXIC POWER OF NORMAL HORSE SERUM.

Since it was my intention carefully to compare the effect of certain methods of treatment upon the production of "antitoxin" in horses' serum, the fact that the blood of these animals in the normal state may possess some amount of this substance,² made it desirable that the serums of my horses should be tested before any treatment was begun. This was done in the following way:—

Some guinea-pigs were given a subcutaneous injection of toxin, just sufficient to cause death, in 2 or 3 days. One animal served as a control; the rest received, mixed with the toxin, different quantities of the serum. The normal serum of Horse No. 1 (the larger and younger animal) was found to possess hardly any power of protecting against the poison. In the first experiment it appeared even to hasten death. But it will be observed that the guinea-pigs died in the inverse order of their sizes; that is to say, those animals which received more toxin died before those which received less.

In the next experiment, the dose of toxin was reduced. Animal No. 3, considerably smaller than the rest, was the first to die, although

¹ By minimal fatal quantity is meant the smallest quantity which will cause death in 48 hours, or rather less. Since this varies with the size of the animal used for the experiment, it is convenient to express the strength of a toxine by the quantity which it would be necessary to use, if the animal weighed 1000 grms.

² Roux, *Ann. de l'Inst. Pasteur*, Paris, vol. viii. p. 616, footnote.

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he received 1 c.c. of the serum. Nos. 1 and 2 lived considerably longer than the control.

TABLE I.

	Weight of Guinea-Pig in Grms.	Quantity of Toxin in C.c.	Quantity of Serum in C.c.	Result.
No. 1, . .	430	·125	3	Death in 50 hours.
No. 2, . .	390	·125	6	„ 51 „
No. 3, . .	365	·125	10	„ 46 „
Control, . .	470	·125	...	„ 55 „

It is a significant fact that of these two animals, which were of exactly the same size, the one which received 2 c.c. of serum lived a day longer than that which received 5 c.c.

TABLE II.

	Weight of Guinea-Pig in Grms.	Quantity of Toxin in C.c.	Quantity of Serum in C.c.	Result.
No. 1, . .	550	·1	5	Death in 64 hours.
No. 2, . .	550	·1	2	„ 89 „
No. 3, . .	350	·1	1	„ 16 „
Control, . .	550	·1	...	„ 42 „

In the third experiment the dose of toxin was not only again reduced, but given in quantities proportionate to the weight of the animal.

TABLE III.

	Weight of Guinea-Pig in Grms.	Quantity of Toxin in C.c.		Quantity of Serum in C.c.	Result.
		Per Kilog.	Actual.		
No. 1, . .	540	·15	·08	10	Death in 116 hours.
No. 2, . .	470	·15	·07	5	„ 176 „
No. 3, . .	580	·15	·087	...	„ 108 „

Again the serum prolonged life, and the smaller quantity acted better than the larger.

The serum of the smaller and older horse (No. 2) gave much better results. In an experiment, strictly comparable with the above (Table III.), 10 and 5 c.c. prevented all local and constitutional effects of otherwise fatal doses of toxin.

TABLE IV.

	Weight of Guinea-Pig in Grms.	Quantity of Toxin in C.c.		Quantity of Serum in C.c.	Result.
		Per Kilog.	Actual.		
No. 1, . .	540	·15	·081	10	Recovered without local lesion.
No. 2, . .	460	·15	·069	5	„
Control, . .	580	·15	·087	...	Death in 108 hours.

Since in all measurements of the antitoxic power of the serum obtained from the horses, after they had been for a longer or shorter time under treatment, the standard quantity of toxin given to the guinea-pigs was ten times as much as would cause death in 48 hours or less, a similar experiment was made with the normal serum of Horse No. 2, in order to compare its power with that obtained from the same animal after a course of injections.

This experiment showed that 5 c.c. per kilog. of this serum was sufficient to prevent all local and constitutional effects of even this large quantity of poison, while 1 c.c. and ·5 c.c. per kilog. merely prolonged life by a few hours.

TABLE V.

	Weight of Guinea-Pig in Grms.	Quantity of Toxin in C.c.		Quantity of Serum in C.c.		Result
		Per Kilog.	Actual.	Per Kilog.	Actual.	
No. 1, .	500	2·0	1·0	5·0	2·5	Recovered without local lesion.
No. 2, .	305	2·0	0·61	1·0	0·305	Death in 51 hours.
No. 3, .	280	2·0	0·56	0·5	0·14	„ 46 „
Control, .	380	0·2	0·76	„ 42 „

When tested against a single minimal fatal dose of living microbes, 5 c.c. of this serum prevented all local and constitutional symptoms,

while the same quantity of the serum of Horse No. 1 prolonged life by a few hours only.

TABLE VI.

	Weight of Guinea-Pig in Grms.	Quantity of Culture in C.c.		Quantity of Serum in C.c.	Result.
		Per Kilog.	Actual.		
No. 1, .	300	·05	·015	5 c.c. of serum of Horse No. 1	Death in 120 hours.
No. 2, .	300	·05	·015	5 c.c. of serum of Horse No. 2	Recovered without local lesion.
Control, .	300	·05	·015	...	Death in 72 hours.

We may conclude from these experiments that the serum of the horse, in its natural state, may possess well-marked antitoxic power.

THE INDIVIDUAL SUSCEPTIBILITY OF DIFFERENT HORSES.

These horses, whose serums showed such very different degrees of antitoxic power, differed greatly in their susceptibility to diphtheria toxins.

A first injection of $\frac{2}{3}$ c.c. of filtrate, mixed with an equal volume of Gram's solution of iodine, produced in Horse No. 1 rather serious symptoms. Profuse sweating and very rapid respiration (60 per minute) for the first few hours, a rise of temperature reaching 104° F. on the following day, and a local swelling $10\frac{1}{2}$ in. in diameter. The whole reaction lasted 3 days.

It was my intention to inject Horse No. 2 with living bacilli only, yet for the purpose of comparing the reaction of the two animals, a single injection similar to that just described was given to this animal. It produced hardly any local or constitutional effect, as might have been expected, seeing that the normal serum of this animal possessed marked antitoxic power.

IMMUNISATION OF THE HORSES.

Horse No. 1 was immunised by Roux's method. The filtrates of old broth-cultures, already described, were subcutaneously injected; at first in minute quantities, and weakened by the addition of Gram's solution of iodine, and afterwards in gradually increasing amounts no longer thus weakened. The effects of the first injection of $\frac{2}{3}$ c.c. already described, were so severe that only $\frac{1}{3}$ c.c. was used on the next occasion, and even this produced a rise of temperature to over 104° F. After this, the quantities were very cautiously increased, and it was

9 weeks before the dose had reached 10 c.c. After a second period of equal duration it had reached 100 c.c. The injections were repeated frequently, each following the preceding one, 24 or 48 hours after the local and constitutional effects of the latter had passed away; that is to say, as a rule, about once, twice, and sometimes three times a week.

After the first 6 months, recourse was had to intravenous injection, in quantities varying from 20 to 300 c.c.

For the treatment of Horse No. 2 a slight modification of Dr. Klein's method was employed. The bacilli from cultures on agar or solidified serum were removed with a platinum wire, and suspended in a small quantity of broth or salt solution, and injected without further delay. The intensity of the effect produced by these injections was regulated by selecting younger or older cultures, and by varying the quantity given. For it is well known that the virulence of a culture of the bacillus of diphtheria diminishes progressively with its age. Accordingly, the first injections consisted of a portion of a culture some weeks old. These were followed by larger quantities of more recent cultures, and later, the growths from several tubes, sown 24 or 48 hours before, were injected at the same time. The injections were repeated about as frequently as those given to Horse No. 1.

With the exception of the first injection already mentioned, nothing but these suspensions of living bacilli were used for the first 6 months. After this, for reasons to be mentioned presently, injections of toxin were given, the first few subcutaneously and the remainder into the veins.

During the whole course of treatment the horses never refused food, nor appeared to suffer anything more than a temporary inconvenience. Weekly measurements showed that they gained in weight at first, and afterward maintained this improvement.

THE EFFECT OF INJECTION OF TOXIN INTO THE HORSE.

The earlier injections caused considerable constitutional disturbance: very rapid breathing, shivering, followed by profuse sweating and diarrhoea. After a few hours these symptoms passed away, giving place to fever, the temperature reaching a maximum of 104° F. on the second, and returning to normal on the fourth day. At the seat of injection, a flat cedematous swelling, with a distinctly marked margin, appeared, reached a maximum of 10 or 12 in. in diameter on the third day, and after that quickly subsided, leaving only at the actual seat of injection a little hard lump, which remained a day or two longer. Subsequent injections produced progressively less result. The symptoms which first failed to appear were the increased rapidity of breathing, the sweating, and the diarrhoea. Very soon the rise of temperature became very slight, and indeed, after the first 7 injections, it was never

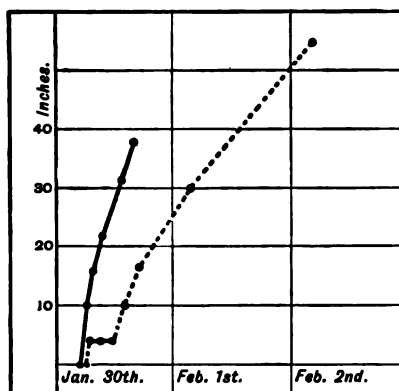
more than 1° above the normal, although the quantity injected was progressively increased. Thus the constitutional symptoms produced by subcutaneous injections after the first few weeks were very slight.

The local reaction did not diminish to the same extent as the constitutional, and the increased quantities of toxin, given as time went on, produced about as much swelling as the smaller quantities did at the beginning. An important difference, however, in the progress and duration of the local reaction was noticed. The swelling appeared earlier, reached its maximum on the evening of the day of injection, instead of on the third day, and no longer persisted three days, but had disappeared, as a rule, on the following morning, or, at most, a hard lump, as large perhaps as a walnut, remained at the seat of injection for a day longer.

Thus, briefly, the change in the reaction to toxin, which took place as the horse gradually became immune, may be summed up by saying that the local reaction became more rapid in onset and of shorter duration, the constitutional reaction becoming less severe.

The change in the course run by the local reaction is to be attributed to an alteration in the local conditions of the part into which injections were made, for up to this time all injections were made into the subcutaneous tissue of the neck, and the whole of this region had been repeatedly subjected to the influence of the toxin. If, now, an injection were made into some new region, such as the subcutaneous tissue of the abdominal wall, a slowly-growing, three-day swelling appeared, similar to that which resulted from an injection into the neck at the beginning of the treatment. This is illustrated by the following experiment: 5 c.c. of toxin was injected in the tenth week of immunisation, under the skin of the neck, and a similar quantity, at the same time, under the skin of the abdominal wall. The swellings which resulted were measured at frequent intervals, and curves representing the areas of the swelling from time to time were drawn.

This experiment was repeated several times, always with the same result.



Abcissae = Areas in Inches.
Ordinates = Days.

FIG. 1.—Curve showing the rate of growth and duration of the swellings which resulted when equal quantities of toxin (5 c.c.) were injected (1) into the neck, where many injections had been made before (straight line), and (2) into the abdominal wall, a new part of the same animal (dotted line).

THE EFFECT OF INTRAVENOUS INJECTION OF DIPHTHERIA TOXIN.

The effect of intravenous injections upon the horse subjected during the previous 6 months to subcutaneous injections of toxin was at first rather severe. Quantities of toxin which, when introduced under the skin, produced no appreciable constitutional disturbance, now caused shivering, dyspnoea, and diarrhoea. Nevertheless, the temperature showed but little tendency to rise. A temperature of 101° F. was the rule when large quantities (about 200 c.c.) were injected.

In the horse previously treated for 6 months with living cultures, intravenous injections of toxin produced similar but more severe symptoms, and the temperature rose considerably, often to between 104° and 105° F. An injection of 60 c.c. of toxin, on June 14th, produced a rise of temperature to between 104°–106° F., although the same quantity of the same toxin, introduced under the skin 4 days earlier, had produced no constitutional disturbance whatever. These symptoms were very transient, and had always disappeared completely on the morning after injection. After a few intravenous injections, the symptoms produced became much less marked, but the febrile reaction remained unaltered.

THE EFFECT OF SUBCUTANEOUS INJECTION OF LIVING MICROBES
INTO HORSE No. 2.

Subcutaneous injections of living diphtheria bacilli produced in Horse No. 2 local and constitutional effects somewhat similar to those seen in Horse No. 1 as the result of injections of filtered cultures. The constitutional effects were, however, much slighter, and the temperature seldom rose more than 1° F. This was probably because the animal possessed at the beginning a considerable degree of immunity. The local reaction also differed in some respects from that produced by injections of filtered cultures. Like the latter, it appeared as a soft, flat swelling of some 10 in. in diameter, which persisted for 3 days and then rapidly faded, leaving a small indurated nodule at the seat of injection, but this nodule often became the seat of a small abscess, which was sometimes absorbed, and at others opened itself spontaneously and discharged its contents. These abscesses were unaccompanied by heat and tenderness, and showed no tendency to increase in size. They never gave rise to any serious inconvenience. I was able to confirm Roux's observation that the pus of these abscesses contain living diphtheria bacilli, and no other microbes.

As in the case of the other horse, injections made at a later period were followed by less severe constitutional disturbance than those which resulted from the earlier injections, and the local reaction appeared earlier, and the greater part of the swelling disappeared in 24

instead of 72 hours. The little abscesses, however, remained for some days, and were more constant after the later injections than after the earlier, probably because larger quantities of culture were injected. This at least was the case when the injections were made into a part which had already been subjected many times to their influence. When injections were made into some new part, the swelling made its appearance slowly, and persisted as long as at the beginning. And if injections of equal quantities of culture were made at the same time into a region where injections had been frequently made before, and into some new part, a difference in the course run by the two swellings which resulted was observed, similar to that which occurred when the same experiment was made with toxins injected into the other horse (cf. Curve No. 1).

METHOD OF MEASURING THE ANTITOXIC POWER OF SERUM.

The antitoxic power of the serum was measured from time to time in the following way:—

The smallest quantity of toxin which, subcutaneously injected into a guinea-pig, could be relied upon to cause death in 48 hours or less, was first determined. A series of guinea-pigs were then injected, each with ten times this minimal fatal dose of toxin, mixed with varying quantities of the serum; the actual amount of toxin given being in proportion to the animal's weight. The injections were made into the subcutaneous tissue of the abdominal wall immediately after the toxin and antitoxin had been mixed together. Those animals which received too small a quantity of the serum either died, or recovered after having had œdema of the abdominal wall. Those which received sufficient serum had no appreciable local or constitutional disturbance. The smallest quantity of serum which would produce this result gave the measure of the strength of the serum. This is expressed as the ratio of this actual quantity used to the weight of the animal. Thus, if the weight of the guinea-pig were 500 grms., and the quantity of serum .01 c.c., the strength of the latter is said to be .01–500 or 1–50,000. In taking this measure of the strength of the serum, I have followed the plan adopted by Roux.

I believe that this method, which has for a long time been used by other observers, may be relied upon to give trustworthy results, for the following reasons:—

The estimation of the minimal fatal quantity of toxin can be made to within one-tenth of its true value: hence measurements of antitoxic power made at different times, and with different toxins, are not affected by these conditions to more than this extent. And, in this research, no attempt was made to measure the power of serum within these limits.

Objection may be raised to the supposition that, in animals of

different size, quantities of toxin proportionate to their weight produce the same effect. To this I would reply, that I have only relied upon this supposition to a very small extent. Throughout, I have used animals of as nearly the same size as possible, and whenever strict comparison was particularly important, animals of exactly the same size and breed have been selected and the experiments made at the same time and with the same toxin.

Further, during my experience of the working of the method, I have been greatly struck with the close agreement in the results obtained.

THE VARIATION IN ANTITOXIC POWER OF THE SERUM OF TWO HORSES, TREATED WITH REPEATED INJECTIONS OF TOXIN AND LIVING BACILLI RESPECTIVELY.

In the following experiments the serum was obtained from blood taken 9, 10, or 11 days after the last injection. The antitoxic power of the serum of Horse No. 1, which was originally 0, was raised in 9 weeks to 1:3000, during which period 12.6 c.c. of toxin in 17 subcutaneous injections had been given. After a second period of 8 weeks, during which 76 c.c. of toxin in 9 subcutaneous injections had been given, it had reached 1:10,000. After a third period of 9 weeks, during which 900 c.c. of toxin in 17 subcutaneous injections had been given, it had increased to 1:15,000.

The antitoxic power of the serum of Horse No. 2, which was

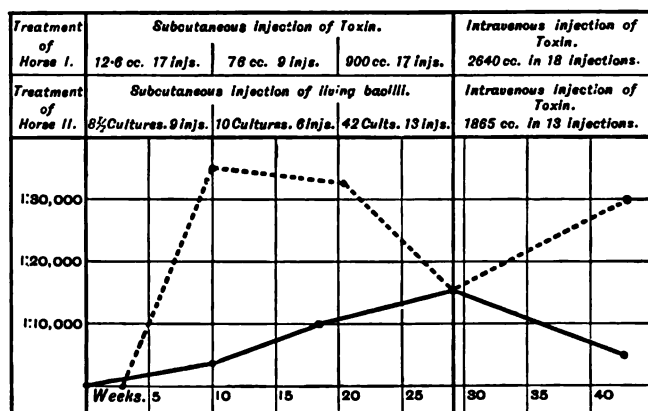


FIG. 2.—Curve showing the variation in antitoxic power of the serum of two horses during 10 months of treatment.
Straight line = serum of Horse No. 1.
Dotted line = serum of Horse No. 2.

originally 1:200, was raised in 7 weeks to 1:35,000, during which period 8½ cultures in 9 subcutaneous injections had been given. After a second period of 7 weeks, during which 10 cultures in 6 subcutane-

ous injections had been given, it showed scarcely any alteration. After a third period of 9 weeks, during which 42 cultures in 13 injections had been given, it had fallen to 1:15,000.

It would be impossible to say whether these results depended upon the methods of immunisation employed, or upon the difference in the original susceptibility of the two horses to the products of the diphtheria bacillus, did not the work of previous experimenters serve as a guide.

Dr. Klein¹ has immunised horses by means of injections of living bacilli, and obtained serum of great antitoxic power in 3 to 4 weeks;² and he has informed me that by this method the antitoxic power of the serum could not be maintained, but fell considerably, after rather a short time. On the other hand, Roux,³ immunising horses by means of toxin, did not obtain serum of this value until a far longer time had elapsed. In one instance, the serum of a horse attained an antitoxic power of 1:50,000 in 87 days, but this seems to have been an exceptionally short time; and in another instance which he gives, this result was only obtained after 9 months.

My experiments, therefore, so far confirmed results already in our possession, and support the opinion that the method of immunisation, by means of living cultures, may be relied upon to give a quicker but less lasting result than the method of injecting toxin.

It is true that Roux⁴ tried the method of injecting living cultures, and found that the serum obtained from the horse so treated had very feeble antitoxic power, but he made no estimation of the qualities of the serum of this animal, until treatment had been continued for 7 months, and, judging by the experience of Dr. Klein and myself, it seems probable that during that time the serum had attained a high degree of antitoxic power, and lost it before the measurement was made.

THE CAUSE OF THE DIMINUTION OF THE ANTITOXIC POWER OF THE SERUM OF IMMUNISED ANIMALS.

The diminution of the antitoxic power of the serum of horses, after a more or less extended course of treatment, has been observed, not only when living bacilli, but also when toxins, were used. Dr. Armand Ruffer has informed me that the first 3 horses which he inoculated lost two-thirds of their antitoxic properties after some time, in spite of repeated injections of filtered toxin. This is a matter of great practical and scientific importance, and demands careful investigation. I observed that Roux found no diminution of the anti-

¹ *Brit. Med. Journ.*, London, 15th Dec. 1894.

² I have measured the antitoxic value of this serum and find it to be 1:50,000.

³ *Ann. de l'Inst. Pasteur*, Paris, Sept. 1894, pp. 615, 636.

⁴ *Loc. cit.*, p. 636.

toxic power of the serum of 2 horses treated by injection of toxin during the space of more than one year.¹ Now the main difference between his method and that used by Ruffer is that the latter made all injections under the skin, in the region in front of the shoulder, while the former, after a period of subcutaneous injection, injected the toxin into the veins. This suggested a possible explanation. It has been pointed out already (pp. 332–335), that subcutaneous injections of toxin or bacilli repeatedly made into the same region of an animal's body, are followed by local lesions which become progressively more rapid in onset and shorter in duration; and that there are good reasons for thinking that these results are due to changes in the tissue into which the injections have been made, for they are not observed when similar injections are made into other subcutaneous regions of the same animal.² It seems probable, therefore, that these tissues, which may be said to be more or less totally immune, have the power of destroying the toxins at the seat of injection, and by so doing of preventing, more or less completely, the constitutional effect which would otherwise be produced by them. If this be the case, then, an animal which continues to receive successive injections into a limited subcutaneous area will cease after a time to be affected constitutionally by them, and its serum will lose the antitoxic properties which it had gained, just as does that of an immunised animal which no longer receives any injections.

This suggestion receives some support from the fact that the diminution of antitoxic power of the serum occurs sooner in an animal treated with bacilli than with toxin, for it is conceivable that a locally immune part would be able to deal with living microbes, and prevent the formation of toxin, more easily than with a large quantity of toxin already formed.³

If this be the true explanation, then a horse whose serum has already lost most of its antitoxic power, while it was continuing to receive subcutaneous injections into the same region of the body, should regain it when injections are made into the veins; and a horse treated by continued intravenous injections should never lose the antitoxic power of the serum. I accordingly proceeded to put this to the test of experiment.

Horse No. 2, whose serum had already lost half its antitoxic power, while it was being treated with subcutaneous injections of living

¹ *Loc. cit.*, pp. 632, 636.

² Similar evidence of local immunity to the *Streptococcus erysipelatis* and its products has been obtained by injecting these microbes, living or dead, or concentrated filtrates of their cultures in broth, into the two ears of rabbits which had recently recovered from erysipelas in one of them.—Cobbett and Melsome, *Journ. Path. and Bacteriol.*, Edin. and London, Nov. 1894.

³ Living diphtheria bacilli are not quickly destroyed in a locally immune part, for, as I have already said, they produce little abscesses, from which they can be obtained alive several days after the injection.

microbes, henceforth received intravenous injections of toxin; and Horse No. 1, whose serum had very slowly but steadily increased in value during the 6 months that it received subcutaneous injections of toxin from this point received the same treatment as Horse No. 2.

In the case of Horse No. 2, the result of this experiment was striking. The effect of 1865 c.c. of toxin¹ in 3 subcutaneous and 13 intravenous injections was to raise the value of the serum during the course of 14 weeks, from 1:1500 to 1:30,000; that is, to almost as great a height as it had previously attained.

The result of intravenous injections into Horse No. 1 does not, however, support the suggested explanation. In the course of 14 weeks, during which 2640 c.c. of toxin were given in 18 injections, the antitoxic power of the serum fell from 1:15,000 to 1:5000.

From these conflicting results no definite conclusion can be drawn, and the question needs further investigation. It is probable, however, that the antitoxic power of the serum of an immunised horse may diminish from two causes—

1. It may diminish, as the immunity of the animal gradually diminishes, when injections are discontinued, or no longer produce any constitutional effect. And this seems to have been the case in the instance of Horse No. 2.

2. Or it may diminish while the immunity of the animal is actually increasing. And this is probably what took place in the case of Horse No. 1. For very large intravenous injections of toxin (300 c.c. of a filtrate, of which the minimal fatal quantity for guinea-pigs was .5 c.c.) produced a rise of temperature of less than 1° F., and no other appreciable effect, and this at a time when the antitoxic value of its serum was only 1:5000, while half the quantity of the same toxin injected into the veins of Horse No. 2, whose serum had a value of 1:30,000, was followed by shivering and a rise of temperature to 104° F.

Thus Horse No. 1 appears to have acquired an immunity which, like that of the hen to tetanus, does not depend upon the possession of antitoxic properties by its serum. If this be true, it would seem that the possession of antitoxic power by the blood serum is a more or less transient phenomenon, and the cause only of an early phase of immunity.

A COMPARISON OF THE NATURE OF THE PROPERTIES OF THE SERUM OF TWO HORSES TREATED BY INJECTIONS OF LIVING BACILLI AND OF TOXIN RESPECTIVELY.

It is well known that the blood serum of animals immunised against several diseases acquires the property of protecting others against these maladies, but that while, in the case of pneumonia,

¹ The minimal fatal dose of these toxins varied from .4 to .6 per kilogram.

cholera, typhoid fever, and several other diseases, such serum protects only against the microbes, and not against their filtered cultures, in the case of tetanus and diphtheria it protects against both. In the latter case, the serum may contain two distinct protective bodies, one of which it has been proposed to call antitoxin, and the other antimycin. It was suggested to me by Dr. Klein that the serum of a horse immunised by means of living diphtheria cultures, freed as far as possible from their toxins, would probably be found to protect better against the living microbes than against their products; while that of a horse immunised by means of filtered cultures would be found to protect better against those poisons than against the living microbes. Accordingly, when the two horses had been treated for 6 months by these two methods, I made an attempt to determine by experiment whether this were so.

TABLES VII. AND VIII.—*Serum of Two Horses treated for Six Months, No. 1 with Filtered Cultures, No. 2 with Living Bacilli, tested both against Toxin and against Living Cultures.*

AGAINST 10 × MIN. FATAL DOSE OF TOXIN.					
EXPERIMENT WITH SERUM OF HORSE NO. 1.					
Date.	Dose of Toxin.		Dose of Serum.		Result.
Weight of Guinea-Pig.	Per Kilog.	Actual.	Relative.	Actual.	
27/5/95, } 545 grms. }	6·0 c.c.	3·27 c.c.	1-25,000	·022 c.c.	Died in 5 days.
1/6/95, } 470 grms. }	6·0 „	2·82 „	1-20,000	·0235 „	Died in 4½ days.
27/5/95, } 550 grms. }	6·0 „	3·3 „	1-15,000	·0366 „	{ Recovered without local lesion.
EXPERIMENT WITH SERUM OF HORSE NO. 2.					
24/5/95, } 490 grms. }	6·0 c.c.	2·94 c.c.	1-25,000	·0196 c.c.	Died in 4 days.
1/6/95 } 440 grms. }	6·0 „	2·64 „	1-20,000	·022 „	Died in 4 days.
27/5/95 } 500 grms. }	6·0 „	3·0 „	1-15,000	·033 „	{ Recovered, having had a like local œdema.
CONTROL.					
21/5/95 } 500 grms. }	0·6 c.c.	0·3 c.c.	Died in less than 47 hours.

TABLES VII. AND VIII.—*continued.*

AGAINST 5×MIN. FATAL DOSE OF LIVING CULTURES.					
EXPERIMENT WITH SERUM OF HORSE No. 1.					
Date.	Dose of Culture.		Dose of Serum.		Result.
Weight of Guinea-Pig.	Per Kilog.	Actual.	Relative.	Actual.	
17/5/95 } 600 grms. }	...	·15 c.c.	1-30,000	·02 c.c.	Died in 5 days.
29/5 A /95 } 650 grms. }	0·3 c.c.	·195,,	1-20,000	·0325,,	Died in 4 days.
29/5 B /95 } 710 grms. }	0·3,,	·213,,	1-15,000	·047,,	{ Local swelling 1 in. diameter, patch of necrosis, $\frac{1}{4}$ in. Recovered.
EXPERIMENT WITH SERUM OF HORSE No. 2.					
17/5/95 } 565 grms. }	...	·15 c.c.	1-30,000	·0188 c.c.	{ Recovered after considerable cedema and separation of a slough, 1 in. in diameter.
29/5 C /95 } 570 grms. }	0·3 c.c.	·171,,	1-20,000	·028,,	{ Local swelling, and little patch of necrosis, not quite so large as in 29/5 B. Recovered.
CONTROL.					
17/5/95 } 520 grms. }	...	·03 c.c.	Died in less than 45 hours.
29/5/95 } 740 grms. }	·06 c.c.	·044,,	Died in less than 42 hours.

The serum obtained from each horse was tested against filtered cultures and against living bacilli. The experiments in which living microbes were used were very similar to those in which toxins were employed, but in this case the standard quantity was only *five* times the minimal fatal dose. The choice of this particular multiple was determined by the fact that, as a rule, about the same amount of serum will protect against this quantity of living microbes, and against ten times the minimal fatal quantity of toxin.

Following Behring, I used for my tests 48-hour cultures of the bacilli in broth. With the object of obtaining cultures of approximately equal strength, equal quantities of broth were employed in tubes of equal calibre. For, as is well known, the rapidity of growth is

influenced by the freedom with which the microbes have access to the air.

The minimal fatal dose of a certain culture having been ascertained, similar cultures were made from the same source, and these were used for the actual experiments. In order that the results might be strictly comparable, corresponding experiments with the two kinds of serum were made at the same time, and with the same cultures.

The following results were obtained:—

Both kinds of serum, when tested against toxin, were found to have the same value, namely, 1 : 15,000. Tested against living cultures, the serum of Horse No. 1 was found to have a value of 1 : 15,000, while that of Horse No. 2 had a value of 1–20,000. An examination of the details of these experiments will show that these figures do not quite do justice to the difference in the properties of these two serums.

I believe that this difference in the power of protecting against living microbes, possessed by two serums of the same antitoxic value, though small, is well beyond the margin of error of the methods employed to determine it, and that these experiments give substantial support to the view that the properties of serum obtained from horses immunised by living cultures and toxin respectively are not identical in their nature.

ON THE VARIATION OF THE ANTITOXIC POWER OF THE SERUM OF AN IMMUNISED HORSE AFTER A RECENT INJECTION OF TOXIN.

The belief is very generally held that antitoxin is a product of the cells of the body. When one reflects that a large injection of toxin probably uses up a considerable quantity of the antitoxin already in the blood, and causes leucocytosis and a fresh production of this substance, it seems reasonable to inquire at what period after an injection we may expect to obtain serum of the greatest value? With the object of answering this question, I took from Horse No. 1 small quantities of blood, 3 hours, 20 hours, 3 days, 6 days, and 11 days after the last injection of 300 c.c. of toxin. This was done at a time when the animal had been treated for 10 months with injections of toxin. I then proceeded to measure the antitoxic value of the serum obtained from these 5 samples of blood. That which was obtained 3 hours and 20 hours after the injection had a value of 1 : 10,000; that obtained 6 days and 11 days after had a value of only 1 : 5000; while that obtained 3 days after was intermediate between these two. This was somewhat unexpected. It is doubtful whether similar results would have been obtained at an earlier period of the treatment of this horse, and more experiments are wanted before we can decide which is the best period to bleed an immunised animal.

CONCLUSIONS.

1. The blood serum of normal horses may possess definite antitoxic power.

2. In the blood serum of a horse immunised by the injection of living bacilli, the property of rendering harmless injections of these bacilli into other animals, predominated over the property of rendering harmless injections of toxin, while these two properties were possessed in equal degree by the serum of a horse immunised by injections of toxin. This observation gives support to the suggestion that there are two different therapeutic agents present in the serum of an immunised animal.

3. The diminution of antitoxic power, which may occur in the serum of horses, in spite of their continuing to receive injections of cultures or toxin, is probably due to one of two causes.

(a) Either to the existence of local immunity in the region into which the injections are made, which, by destroying *in situ* the material injected, allows little or no constitutional effect to be produced upon the animal.

(b) Or to the fact that the immunity, which the horse ultimately acquires as the result of treatment, is like many observed instances of natural immunity, independent of the possession of antitoxic properties by the serum of the animal.

4. Finally, I would remark that the results of the experiments described in this paper seem to indicate that the best and quickest method of obtaining serum of antitoxic value from the horse would be to commence with injections of living bacilli, and after a period of perhaps 5 or 6 weeks to proceed with intravenous injections of filtered cultures.

UPON GENERAL INFECTION BY THE BACILLUS PYOCYANEUS IN CHILDREN.

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THE observations of Bouchard, Charrin (¹), Ruffer, and others have shown that the *Bacillus pyocyaneus*, obtained primarily as a contamination in the pus of wounds, is very definitely pathogenic for certain of the lower animals, notably rabbits. While occasionally met with in association with other microbes, it has been but rarely found as a primary cause of disease in man.

The cases here related being, beyond doubt, of this nature are therefore of more than usual interest.

CASE 1.—C. E., an illegitimate male child; born on 24th October 1893, in the Montreal Maternity Hospital, and admitted on November 17th to the Montreal Foundling and Infant Nursery with his mother, a Swedish woman in good health. He was entirely breast-fed, and gained steadily until the twenty-second week of age, when he became restless and ill, and began to lose weight. These indefinite symptoms, for which no cause could be assigned, continued for 5 weeks, when diarrhoea with green stools set in, accompanied by fever (99°–100°), abdominal pain and tenderness, but no tympanites. About the first week of May a group of half a dozen purple papules, 3–7 mm. in diameter, appeared on the abdomen on each side, midway between the umbilicus and the flank. The skin over the abdomen was relaxed, dry, and wrinkled. The child was then in a low, depressed state, lying listlessly in his mother's arms, frequently moaning, especially when disturbed. During the following fortnight numerous papules appeared, extending up and down from the original groups and across the hypogastrium, in the form of an irregular horseshoe; still later many of these became confluent, and others appeared on the abdomen and chest. By the 18th the depression became extreme; the green stools continued, although the abdominal pain and tenderness had ceased. The abdominal facies was very marked. The child could not, or would not, move his limbs. The legs were flexed upon the thighs and the thighs upon the abdomen, and any attempt to straighten them out caused the child to moan; when released they at once returned to their former position. The papules now, for the first time, were seen on the thighs and shoulders.

¹ Since this paper was put into type Professor Adami has written to say that Dr. Williams has succumbed to an attack of blood poisoning, contracted during the performance of his pathological work. Dr. Williams was an earnest investigator, and there seems to be little doubt that overwork left him very susceptible to infection.—ED.

On the 22nd there was a very profuse epistaxis, followed by a refusal to take food. On the 23rd the superficial abdominal veins became distended. On the 25th subcutaneous hæmorrhages occurred from between the toes and from papules on the right thigh and back. Nearly all the spots were now of a darker colour. The next day a slight purulent discharge from the left ear was observed. The child became comatose, and died on 27th May, two months from the onset of the illness. The case was looked upon as one of purpura hæmorrhagica of septic origin.

An *autopsy* was performed shortly after death. The body was not markedly emaciated, there being a fair quantity of subcutaneous fat. Sections through the blue spots showed deep pigmentation of the skin and infiltration of the subjacent fat and loose tissue with dark blood.

The muscles and the thoracic and abdominal organs, except the spleen, were very pale. The heart was almost empty, save for a little fluid blood in the left auricle. The spleen was firm and of a deep crimson colour, the capsule firmly adherent, and the Malpighian bodies distinct. The kidneys were large, and on the inferior surfaces there were two or three small purplish spots, over one of which the capsule was puckered and thickened. On section these resembled small, firm infarcts. There was no sign of any marked inflammatory lesion in the intestines, though the mucous membrane was thickened.

Microscopic examination of the kidneys, liver, and spleen, showed that many of the capillaries were blocked with emboli, formed by minute bacilli; in some instances the micro-organisms had passed through the walls of the vessels, infiltrating the surrounding tissue. There was a slight generalised parenchymatous nephritis, the tubules, especially in the convoluted portion, being swollen and cloudy and filled with granular débris. The liver cells were also irregular and cloudy.

Minute portions of tissue were removed from the spleen pulp, and from one of the hæmorrhagic spots on the kidney, and put into culture tubes of gelatine (containing 1 per cent. of Mercks' peptone and 0·5 per cent. of sodium chloride), and of agar-agar (1 per cent. peptone, 1 per cent. glycerine, and 0·5 per cent. sodium chloride). These tubes were kept at the ordinary temperature of the room, and on the sixth day minute creamy white striæ or films were seen upon the surface of the media. The tubes were then placed in an incubator, when by the following observations the growth was found to be that of the *B. pyocyaneus*.

We shall enter at some length into the cultural characteristics of the organism isolated by us, inasmuch as the growths did not wholly correspond with those described by other writers on the *B. pyocyaneus*. To this point we shall refer later.

On *agar-agar* the growth was moist, spread rapidly along the needle track and radiated over the surface of the medium, which soon became a fluorescent light green, usually with a slight bluish tinge at the edge of the growth. Later, the agar became a deep grass-green, and finally a nut-brown.

Eight weeks later some agar tubes prepared without glycerine were inoculated from the first ones. The growth was at first the same, but after two days the edges became moist, and soon the moisture ran to the bottom of the tube where the growth rapidly continued. Upon the surface of the primary growth minute stellate spots, with a dry metallic lustre appeared, and rapidly coalesced and spread until the entire surface presented the lustre.

On the *gelatine* a thin greyish film appeared on the surface, while the upper portion of the medium rapidly liquefied. This film became thick and floated on the surface, while colonies sank and continued the liquefaction. The liquefied portion was reddened with a greenish cloud in the upper part near the surface. When the tube was shaken the entire fluid became a bright opalescent bluish-green. This colour faded, but could be observed near the surface after standing. It could be at any time reproduced by shaking. Later

the gelatine all became liquefied and of a reddish colour, but when shaken turned a grass-green. The growth sank to the bottom of the tube. In an old tube no green could be produced, and the colour became a deep brownish-red.

Cultures made from papules on the skin remained sterile, in consequence, perhaps, of the presence of the bichloride of mercury with which the skin was washed, and others from the contents of the small intestines contained intestinal bacteria but no *B. pyocyaneus*.

On the tenth day after the first series of cultures had been made, a second series of tubes of beef-broth, and of egg-glycerine were inoculated from the agar and gelatine which had shown the characteristic growth.

In *alkaline beef-broth* (1 per cent. peptone and 0.5 per cent. sodium chloride), tubes placed in the incubators at 37° C., a greyish film appeared on the surface in 24 hours. On shaking, the entire broth became cloudy and of a bluish-green colour, which later turned to a grass-green. The colour would fade and reappear as in the gelatine.

On *egg albumen* a creamy bluish-white growth spread rapidly along the needle track. The medium became first a bluish-green, and the liquid formed was of an opalescent or cloudy blue.

Later, the egg was of a deep blue colour, and the liquid was green at the upper part and red at the bottom of the tube. On shaking, the egg dissolved, and all became a dark green. In old tubes the colour was generally a deep greenish-blue.

On *egg glycerine* (1 per cent. glycerine) the growth resembles that on egg. In 36 hours the medium became a beautiful bluish-green, which in the course of 7 days turned to a deep blue colour, except at the edge of the growth. The liquefied portion was blue and cloudy. In old tubes, when all was liquefied, the colour slowly became red.

On *potato*, in 15 hours, the growth spread from the needle track over the surface. The growth was moist and fawn-coloured, while the potato became a bright green. Later the growth became dry and shiny, and, like the potato, turned a dark dirty green.

In *broth containing 0.7 per cent. boric acid*, many long bacilli were seen in active growth, some were curved, others had slightly clubbed ends. No colour was produced.

On *beef-broth with 5.5 per cent. peptone*, the growth was rapid, the bacilli dividing and forming short chains, while many became from three to six times longer than in the broth with 1 per cent. peptone.

All the cultures after a few days emitted the peculiar odour of trimethylamine, characteristic of cultures of *B. pyocyaneus*.

The blue crystals of pyocyanine were readily obtained by adding chloroform to the alkaline fluid cultures and evaporating slowly to dryness.

If to any of these cultures chloroform was added, it assumed a beautiful Cambridge-blue colour. On rendering the fluid acid by dilute sulphuric acid the blue coloration of the chloroform disappeared, and the supernatant fluid became pink. The addition of ammonia again rendered the chloroform blue.

To inoculate a rabbit, an agar-agar roll tube was prepared, and from it a minute colony was removed and placed in a tube of beef-broth. Twenty-four hours later, when the broth showed a faint greenish colour, 0.5 c.c. was injected into a vein in the ear of a healthy rabbit. In less than 24 hours the animal was unable or unwilling to move, and the hind-legs became stiff.

A slight diarrhoea set in, all the muscles stiffened, and death occurred 40 hours after inoculation. On examination, small punctate red hæmorrhages were seen in the mucous membrane of the stomach. There were none in the intestines or on the skin.

The urine contained a small quantity of albumen.

Microscopic examination of the organs showed conditions similar to those met with in the organs of the child, though the vessels were somewhat more markedly affected.

Culture tubes prepared from the blood, liver, spleen, kidney, and urine showed in 24 hours the same growth and colour production as those from the child, but of a more pronounced type. The growth was more rapid and the colour more durable.

It was noted that although the original cultures from the rabbit were afterwards exposed to diffused sunlight for about five hours daily for nearly three weeks, their virulence had not diminished.

It is true that our test-tubes were of fair size (18 mm. in diameter), and that through the growth and colour production the broth was opaque. Nevertheless, bearing in mind the outcome of recent researches, we had not expected to find the strong sunlight of a Montreal summer absolutely without effect upon the virulence of the bacillus.

CASE 2.—C. B., an illegitimate female child; born in the Montreal Maternity, 8th April 1894, was nursed by its mother and left by her in the nursery on 7th June. The infant was poorly nourished, small and thin, weighed 7 lb. 4 oz., and had a purulent discharge from both ears. She was fed on a mixture of Nestlé's Food, peptonised milk and cream. During the first week there was a gain of 4 oz., and the discharge from the ears ceased. After that she steadily failed, the stools became frequent, green, and very offensive.

Treatment with lavage and salicylate of bismuth was of no avail. There was a general lividity of a most pronounced type of the whole body, with a dozen or so pustules full of yellow pus on the head, but no purpuric spots or cutaneous hæmorrhages were ever observed.

Two days before death the lividity became extreme, and there was general rigidity of the muscles. The child died on 1st July, three weeks after admission.

Two days before death tubes of beef-broth and gelatine were inoculated with the pus from one of the pustules on the head, and from some blood taken from the tip of a finger. In neither case did the characteristic growth of *B. pyocyaneus* appear.

At the *autopsy*, three hours after death, the body was found to be extremely emaciated, little or no subcutaneous fat being present.

All the abdominal and thoracic organs were pale.

The mucous coat of the stomach and intestines was thickened, and presented a few scattered punctate hæmorrhages, especially in the small intestine. The bladder was distended with pale urine.

Culture tubes of gelatine were inoculated from the kidney, spleen, liver, heart blood, and urine, which, with the exception of the two latter, presented pure growths of the *B. pyocyaneus*, the appearance being identical with those seen in Case 1. The heart blood and the urine remained sterile.

Two weeks later, from the growth from the spleen a roll tube was prepared, and from a colony in it a tube of broth was inoculated.

After two days, 0.5 c.c. was injected into a vein of a rabbit's ear. The animal died 19 hours afterwards, and, at the post-mortem examination, hæmorrhages were seen in all the organs, but were most marked in the stomach (especially at the cardiac end) and kidneys. Cultures taken from the organs presented the green coloration, but much less marked than in those taken from the child.

The cultures from these cases have been compared from day to day with cultures from bouillon tubes of *B. pyocyaneus* brought from Cambridge in 1892. The only difference has been in the intensity of the colour, which, perhaps on account of the greater age, has been less pronounced in the Cambridge growths.

In connection with these cases two others may be mentioned which occurred in the same ward. These presented very similar symptoms, but the bacillus under discussion was found in only a single part of the body, and in association with other organisms.

CASE 3.—M. T., an illegitimate female child; born at the Western Hospital on 20th February 1894, was admitted to the nursery on 13th March in good condition, having been nursed by its mother up to that time. After a month of good health it began to waste, and have green motions, diarrhoea, and fever. The skin soon presented marked lividity, and numerous pustules and subcutaneous abscesses developed all over the body, but especially on the head and back. The large majority of these contained pus of a yellowish colour, but in a few it was brown, and from these latter, growths on nutrient media were obtained, which presented all the characteristics of the *B. pyocyaneus*. The *Staphylococcus pyogenes citreus* was also isolated. The child died on 24th June, but unfortunately no autopsy was obtained.

CASE 4.—M. R., an illegitimate female child; born at the Western Hospital on 31st May, and admitted to the nursery 20 hours later. Partly wet-nursed, it maintained its weight (111 oz.) for three weeks, until a purulent discharge from both ears was noticed; this was checked in four days. The symptoms which then followed were diarrhoea, wasting, general lividity, slightly elevated temperature (100° F.), and cold extremities. Three days before death numerous small hæmorrhagic spots of a port-wine colour appeared on the abdomen and back, a large patch over the right scapula, and a few on the head, shoulders, and legs. A large bed sore developed over the sacrum. The lower limbs became flexed and rigid, the child crying when they were straightened. Temperature fell to 95° F. The child died on July 23rd.

At the autopsy, performed two hours after death, all the organs were found to be very pale, except the spleen, which was large and dark. There were signs of intestinal catarrh, but no hæmorrhages. There were no lesions in any other organs. Intestines full of yellow fæces, bladder empty. Cultures from the various organs remained sterile, except those from the contents of the cæcum, which, on the following day, showed a green colour, and from amongst the intestinal bacteria the *B. pyocyaneus* was isolated.

Three cases have been reported of the discovery of this bacillus in association with symptoms which were in many respects similar to those in the first two cases here reported.

Ehlers⁽²⁾ reports the following: A brother and sister, respectively 11 and 12 years old, after a prodromal period, suffered from fever, profuse diarrhoea, enlargement of the spleen, mental depression, and prostration. They were thought to have either typhoid fever or cerebro-spinal meningitis.

About the twelfth day an eruption of papules occurred on the surface of the body and limbs, especially anteriorly. These soon became pustular and bullous, like ecthyma, the bullæ containing a blue fluid, and ulcers were formed, with their borders hard and pigmented by hæmorrhages.

One of these children died, and from the pustules, spleen, blood, etc., the *B. pyocyaneus* alone was separated.

Neumann⁽³⁾ reports a case of a child 13 days old, which had symptoms of enteritis, with icterus, petechiæ, and hæmorrhages from the mucous surfaces.

He found, after death, ecchymoses into the skin and mucous membrane of the intestines, a swollen spleen, and parenchymatous degeneration of the kidneys. Microscopically, there was an interstitial hepatitis, which was supposed to be due to syphilis. In the liver and spleen were recognised bacilli, which

had produced no apparent change in the tissues, and from these organs he obtained pure cultures of the *B. pyocyaneus* (B).

Though these three cases seem to be the only ones recorded in which the *B. pyocyaneus* has been found alone, there are numerous instances in which it has occurred in cases of septic infection in association with other micro-organisms.

It will be seen, upon studying the cultural peculiarities of the germ isolated by us, that the results of the cultivation of this bacillus, although closely resembling, for the most part, those described by Gessard (⁴), occasionally showed variations.

For instance, while the growth on agar containing glycerine corresponds to that of Gessard, the growth on agar without glycerine gave a metallic lustre, which appears to be the same as that obtained by H. C. Ernst (⁵), with the *B. pyocyaneus pericarditidis*. On beef-broth, containing 5 per cent. peptone, we also found that the bacillus grew to a greater length. This is another point upon which stress is laid by H. C. Ernst in his endeavour to establish a separate species.

From time to time slight variations resembling those of the *B. pyocyaneus* of P. Ernst (⁶) have been noted.

From these facts it seems probable that these bacilli are capable of many variations in form and colour production, according to their environment, and that further experiments will prove Gessard to be correct in his opinion that they are but varieties of races of the same bacillus.

Since our attention has been drawn to the presence of the *B. pyocyaneus* 10 infant bodies have been examined (post-mortem), with the result that in two a general distribution of the *B. pyocyaneus* has been found, and in one the bacillus was found present in the alimentary canal only. This fact, taken in connection with the observations of Neumann and Ehlers, shows clearly that the infant organism is susceptible to the invasion of the bacillus.

The discovery of this micro-organism alone, associated with a train of symptoms closely resembling in so many respects the disease, produced experimentally in rabbits by Ruffer (⁷),—who found present emaciation, diarrhoea, fever, muscular disorders, albuminuria, and hæmorrhages—the first and last being the most prominent in cases running a slow course, symptoms which are by no means uncommon in infants, especially when they are gathered together in institutions,—indicates that the bacillus is distinctly pathogenic at that early age.

We therefore conclude that in these two cases we have obtained a disease due to the growth of the *B. pyocyaneus*, that, in fact, we have here two typical examples of this very rare condition of true pyocyanic disease, or, if the term be admissible, cyano-pyæmia.

How this disease has originated, that is to say, by what channel the bacillus has gained admission into the organism in these two cases, must still, we think, be a matter of uncertainty.

It is possible that the entry was through some cutaneous lesion, and this would bring these cases into line with those in which the bacillus is found in association with other septic micro-organisms in wounds. But it must be acknowledged that in none of our instances was there any antecedent cutaneous disturbance recognisable or noted.

As regards this point, while Bouchard ⁽⁸⁾ has held that intravenous injection is necessary to produce a general infection by the bacillus, Ruffer seems conclusively to prove that rabbits may be infected by subcutaneous injection.

Another point of entry that might be considered is through suppurative disease of the middle ear. That this is a possibility is shown by the observations of Dr. F. R. Blaxall ⁽⁹⁾, who found *B. pyocyaneus* in the ear discharge occurring as a complication in scarlatina.

In our second case the infant, when it entered the nursery, had a purulent discharge from both ears. This ceased before the progressive emaciation characteristic of the pyocyanic disease began to show itself; it is possible, however, that this was the starting-point of the malady.

It must not be left out of account that the alimentary tract may have been the point of invasion. In favour of this view it is to be noticed that in two instances we have discovered the bacillus in the contents of the small intestine; in one of these in association, in the other unassociated, with the general disease; that Booker ⁽¹⁰⁾ has discovered the *B. pyocyaneus* in the intestinal tract of a number of children examined with reference to intestinal bacteria; and that during the first few weeks of life the digestive functions of the infant, especially if artificially fed, are but feebly established, and any impairment of these imperfect secretions renders the food taken a most favourable nidus for the production of abnormal fermentation and the growth of bacteria.

In all the cases here reported signs of gastro-intestinal irritation were present for some time before the skin lesions appeared, but whether this was the result of the intoxication by the bacillus, or only favoured its growth, we have no means of determining, for bacteriological examinations of the stools were not made at the time.

A careful bacteriological investigation of all cases of so-called "infantile marasmus," especially when complicated with skin eruptions (whether petechial or pustular), diarrhoea, fever, and muscular disorders, may demonstrate that the distribution of this micro-organism is much more widespread, and much more disastrous in early life than is generally believed. The close resemblance of the symptoms of Case 1, to those generally described in text-books as purpura hæmorrhagica, suggests a causative relation between the bacillus and that lesion.

We are aware that several species of bacteria have been described in connection with purpura hæmorrhagica; the most that we wish to suggest is, that in children the *B. pyocyaneus* may more frequently

be the causal agent in the production of this lesion than has been heretofore held.

In conclusion, we wish to express our sincere thanks to Professor Adami for his assistance and advice throughout the study of these cases.

Note.—This paper was completed in August 1894. Unfortunately the copy first sent to the *Journal* was lost in transit.

[The first copy was announced by letter, but never arrived.—Ed.]

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THE GROWTH OF CHOLERA (AND OTHER) BACILLI IN DIRECT SUNLIGHT.

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IN a recent paper¹ I was able to show that the rate of destruction of tetanus bacilli (in cultures in liquid media) varied when exposed to sunlight in the presence of air, according to the relation of the surface area to the volume of the culture.²

It was found that when equal volumes of the same culture were so exposed, destruction was more rapid in those of large area.

Other observers had noticed that on exposing plate-cultures to the action of the sunlight, whilst the bacteria on the surface were destroyed, those situated more deeply in the culture medium were still capable of development.³ For this reason, Marshall Ward, in his experiments, first poured out his agar-agar plates, and then inoculated them, after they had set, by smearing the surface with bacteria by means of a camel's-hair brush. He was thus able to obtain complete destruction of the bacteria in these cultures, or portions of cultures, which had been acted on by the sunlight.

The growth of colonies in the depth of the medium, after exposure of the plates to direct sunlight, had received several explanations, none of which seemed satisfactory. Thus it had been suggested that the bacteria in the upper parts shaded those situated more deeply, and in this way permitted them to grow. These observations of others, together with those made by myself, led me to think it possible that the harmful action of sunlight was appreciable only at the surface of cultures—in other words, in a region where the oxygen was most abundant.⁴

¹ "Some of the Effects of Sunlight on Tetanus Cultures," *Journ. Path. and Bacteriol.*, Edin. and London, Nov. 1894.

² See also Dieudonné, *Arch. a. d. k. Gendtsamte.*, Berl., 1894, bd. ix. s. 537, etc.

³ Marshall Ward, *Proc. Roy. Soc. London*, 1893, vol. liii. p. 310; and in a lecture given before the Cambridge Philosophical Society, 1894.

⁴ Downes and Blunt, *Proc. Roy. Soc. London*, vol. xxvi. No. 184, Dec. 6, 1877; Roux,

In order once more to test this theory, four cultures of the vibrio of Asiatic cholera were made. Each tube contained 10 c.c. of ordinary peptonised beef-broth, and the depth of liquid was the same in all, namely, 9 cm.

The upper part of one tube was enveloped in black paper, so that all of the liquid except the surface and that portion immediately beneath, for a distance of 1 cm., was exposed to the action of the sun (the surface, for a depth of 1 cm., protected, and the lower 8 cm. exposed). The second was covered with black paper, from the bottom to within 1 cm. of the surface (the surface and upper 1 cm. exposed, and lower 8 cm. protected). The third was encircled by black paper, 5 cm. broad, so that a depth of 2 cm. at the surface, and the same at the bottom were left exposed to the sun's rays. The fourth tube was left quite unprotected, and served as a control. All of the tubes were plugged with cotton-wool, and were in every other way ordinary aerobic cultures. Each was inoculated with the same amount (1 wire loop) of a fresh bouillon culture of the comma bacillus. They were then immediately suspended from a glass rod, and placed against a window, through which the brightest summer sun was shining.

A white cotton screen was placed behind them, with its upper border resting against the window above the tubes, so that they were enclosed in a space formed by the window, the screen, and the table on which the screen stood. Curtains at each end of the screen completed the box-like arrangement. A thermometer placed in a test-tube of water, and suspended in the same position and manner as the culture tubes, indicated a temperature which varied from 25° C. to 37° C., which was higher than elsewhere in the room, the effect of the screen being to raise the temperature of the air between it and the window.

The tubes were exposed in this way for 9 hours, the only change being to move them to another window at noon, so that they might never be out of the direct sunlight.

In using an aerobic micro-organism, like that of cholera, the expectation was that destruction of the bacteria would take place in those cultures in which the surface was exposed to the sun's action, whilst growth would occur in the one tube in which the surface was protected by the black paper.

The cultures were examined at short intervals, and it was noticed at the end of about 5 hours that they had lost their transparency. After 9 hours they were all very markedly turbid. This turbidity was, on microscopic examination, seen to be due to a plentiful and characteristic growth of the vibrio.

Neither on macroscopic nor on microscopic examination could any

Ann. de l'Inst. Pasteur, Paris, 1887, p. 445; Momont, *Ibid.*, 1892, vol. vi. p. 21; Richardson, *Proc. Chem. Soc. London*, 1893, p. 121; Frankland, "Micro-Organisms in Water," p. 380, etc.; Dieudonné, *op. cit.*

difference be observed in the rate or character of the growth in the four cultures. Abundant growth had taken place in all.

It seemed now necessary to determine the influence of the depth of the medium on this process, and experiments were made with this end in view.¹

For these experiments the tubes were unprotected by black paper, and the sun was permitted to fall freely on all parts. Clear, nearly colourless, beef-broth or peptone solution was placed in the tubes in depths varying between 1 and 9 cm. Each tube was inoculated with the same amount (1 wire loop) of a fresh broth-culture, and immediately all were placed in the window as before.

It will be seen that the tubes containing the small amounts of medium, contained, initially, more bacteria in proportion to the contents; or, in other words, the bacilli were *relatively* more numerous, as the amount used for inoculation was the same for all.

The tubes were exposed, as before, to continuous sunshine for 9 hours. In those tubes, in which there was a sufficient depth of fluid, growth took place in 4–5 hours, and at the end of 9 hours the turbidity was well marked. In the tubes containing very little broth no growth could be observed at the end of the experiment, nor on keeping in the incubator did any appear.

In the cultures in which the depth of liquid was 2–4 cm., although no turbidity could be observed at the end of the exposure to sunshine, growth appeared later.

It was found possible, then, to draw roughly a line of demarcation between those tubes in which the bacteria would invariably be killed and those in which growth would occur.

In tubes containing a depth of 2 cm. the bacteria were usually completely killed, whilst in those of 1·4 cm., and less, complete destruction took place in every experiment.

On one occasion, one tube, in which the bouillon was 1·7 cm. in depth, showed no signs of growth after 9 hours' exposure to the sun, but later a typical growth appeared. In the same experiment the bacteria in a tube, which contained medium to the depth of 1·1 cm., were completely destroyed.

These results were verified by making experiments with cultures in solid media, in which the bacteria were either destroyed or grew in the original position in which they had been placed. Stab-cultures, in clear transparent agar-agar, gave, at the end of 9 hours' exposure to bright sunlight, a well-marked growth along the entire line of puncture, with the exception of that part at the surface.

These cultures resembled in appearance anærobic stab-cultures, and were exposed to the sun for several days in succession, being kept at

¹ In connection with this question see Buchner, *Arch. f. Hyg., München u. Leipzig*, 1893; Procacci, *Ann. d. Ist. d'ig. sper. d. Univ. di Roma*, 1893, vol. iii. p. 437; Arloing, *Arch. de physiol. norm. et path.*, Paris, 1886, vol. iii. No. 3; Frankland, *op. cit.*

night in the ice-cupboard, which prevented development between the times of exposure. At the end of the last exposure, though no growth had appeared on the surface, the cultures were placed in a dark incubator, when the growth spread from the needle-track and covered the whole surface.

This would seem to indicate that although the sunlight had been quite capable of preventing any growth on the surface, it had not done so by rendering the culture medium unfit for development.¹

Cultures made by smearing with cholera the sloped surfaces of nutrient agar-agar were not only prevented from growing by the sunlight's action, but the bacteria were entirely destroyed, so that no growth occurred when they were afterwards incubated in the dark. This was also true of plates made and treated in the same way.

When agar-agar plates were made in the ordinary way, no colonies appeared on the surface, either during the time of exposure to sunlight or later. Those bacteria in the depth of the medium grew as if under ordinary circumstances in the dark incubator.

This was very well shown by inoculating a tube of molten agar, with which a plate was poured. After the plate had set, another tube of molten agar was poured over the surface. Such plates, on being suspended in the same manner as the tubes before described, gave most luxuriant growths in the brightest sunlight.

When it was ascertained beyond all doubt that under certain conditions (*i.e.* when the liquid medium was of sufficient depth), the vibrio of cholera was capable of growing vigorously, when exposed to strong sunshine in the presence of air, it seemed desirable to know whether the bacteria so grown were affected in regard to virulence.²

Freshly-inoculated tubes of broth or peptone solution were hung in the window, in the way before described, for a time varying from 5–9 hours. During the night the cultures were kept in an ice-cupboard; and on the following morning, from the tube containing the greatest depth of medium (usually 5–9 cm.), a fresh series was inoculated and suspended in the sunshine. This was repeated six times, and during the whole period continuous sunshine prevailed, so that at the conclusion there were obtained vibrios of cholera which had been grown for six generations in the bright sunshine. The temperature registered by the thermometer, hanging in a test-tube of water, and suspended with the cultures, varied usually between

¹ See Roux, *Ann. de l'Inst. Pasteur*, Paris, 1887, vol. i. p. 445; Pansini, *Riv. d'ig. prat. e sper.*, Napoli, 1889; "Azione della luce solare sui micro-organismi"; Janowski, *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1890, bd. viii.; Ward, *op. cit.*; Dieudonné, *op. cit.*, p. 405.

² Against this the objection might be brought forward, that a transference of even very attenuated cultures of the cholera vibrio to fresh agar-agar might have restored their virulence. There seemed, however, no other method in which comparison could be satisfactorily made, since neither ordinarily incubated broth-cultures nor insolated broth-cultures developed sufficiently in 9 hours for inoculation experiments.

22° C. and 35° C., although on the second day, for about 2 hours, it remained at 41° C.

The cholera vibrios used for these experiments had been isolated but a few days before from a case of the disease occurring at Bürgeln.

These cholera bacteria, after growing for six generations in the direct sunlight, were transferred to agar-agar, and the virulence compared with similar cultures made from the same cholera which had been kept in the form of broth-cultures in the dark.

The test cultures used for inoculation into animals were grown for 22 hours at a temperature of 37°·5 C., on the sloped surface of agar-agar (so inoculated that when grown the whole surface was covered).

The whole of the growth was scraped from the surface of the culture and suspended in 10 c.c. of sterile bouillon, and injected into the peritoneal cavities of guinea-pigs as follows:—

No.	Weight in Grms.	Dose of Suspension.	Material Injected.	Temperature.				Remarks.
				12 A.M.	2 P.M.	4 P.M.	7 P.M.	
1	600	1 c.c.	Cholera, which had been kept and grown in dark.	Received Inoculation.	40·0	39·3	36·5	Dead next morning.
2	545	2 c.c.	„		39·1	39·7	34·3	„
3	460	3 c.c.	„		39·4	37·3	34·5	„
4	665	1 c.c.	Cholera, which had grown for six generations in sunlight.		39·8	39·2	37·8	Very ill next morning; died 29 hours after inoculation.
5	595	2 c.c.	„		39·5	39·1	36·8	Dead next morning.
6	475	3 c.c.	„		37·5	37·4	34·8	„

As will be seen by the foregoing table, there was very little, if any, decrease¹ in the virulence of those cultures which had been grown in sunlight for six generations. The animal which survived longest was the largest of the series, and he died within 30 hours after inoculation of so small a dose as one-tenth of a culture.

Although no exhaustive experiments were made, it was found that *Bacilli prodigiosus*, *B. typhi*, and *B. coli communis* grow quite well in sunlight, and they were found to behave in the same way as the

¹ Palermo working differently, *i.e.* exposing fully-grown cultures for a few hours only, obtained evidence of attenuation; *Ann. d. Ist. d'ig. sper. d. Univ. di Roma*, 1893, vol. iii. p. 463.

comma bacillus, developing quite vigorously at the end of 9 hours, when in a depth of liquid of 5 cm., and at a temperature of 25° C.–37° C. *B. prodigiosus*,¹ under these circumstances and at this temperature, did not form any colour, though this appeared later when left in the light of an ordinary room for a few days at the room temperature. No experiments were made to determine whether the virulence was affected or not in these cultures.

As might be expected from these experiments, and those mentioned in a former paper, it was found that cultures of the *B. tetani*, when freshly inoculated and sealed up in an atmosphere of hydrogen, were not destroyed, but, on the contrary, grew luxuriantly, even in the brightest sunshine. Moreover, cultures so grown were not at all diminished in virulence.

In tetanus-cultures grown in the direct rays of bright sunlight, suspended in a window but without the screen behind them, the bacilli seemed almost asporogenous. This was seen to be due to the comparatively low temperature (15–22° C.) at which they had been grown, since, on raising the temperature, spore formation immediately commenced.²

The effect of sunlight on cultures of cholera bacilli may be said to be twofold—

1. It is destructive to the bacteria on which it falls, *if they be in free contact with the air*.
2. It aids the growth of those bacteria on which it falls through its heating power, if the bacteria be *not* in free contact with the air.

With reference to the first conclusion, the destructive power of sunlight has been pointed out by so many observers that it scarcely needs confirmation. In my experiments the cultures in solid media, in which the bacteria on the surface were killed, and that series in which total destruction of the micro-organisms in very shallow liquid cultures occurred, may be quoted as additional proof of the fact.

That sunlight aids the development of those bacteria which are not freely exposed to the air is shown—

(a) By the growth which takes place during the time of exposure in liquid cultures, where the depth is greater than 2–4 cm.

The same is true of cultures in solid media under the same conditions, with the exception that the accompanying destruction is more strictly limited to the surface, because of the limitation of movement, the bacteria remaining *in situ* in solid cultures; whereas in liquid media, from a variety of causes, such is not the case.

¹ Gaillard, "De l'Influence de la lumière sur les micro-organismes," Lyon, 1888; Pansini, *op. cit.*; Laurent, *Ann. de l'Inst. Pasteur*, Paris, 1890, vol. iv. p. 478; Dieudonné, *op. cit.*; d'Arsonval and Charrin, *Compt. rend. Soc. de biol.*, Paris, 1894, vol. cxviii. p. 151.

² It may be here mentioned that, when the white screen was placed behind the exposed cultures and the temperature thus raised, growth occurred much more quickly than when the screen was not employed.

(b) By the free growth that takes places in anærobic cultures in an atmosphere of hydrogen, when exposed to the strongest sunlight.

When considering experiments made to determine the effect of direct sunlight upon freshly-inoculated cultures of ærobic micro-organisms, it seems safe to assume that two processes are going on at the same time, namely, a destructive action at the surface and growth in the depths of the medium.

If the medium be deep, the growth outstrips the destruction, and, after a short time, turbidity may appear just as in cultures grown under ordinary circumstances.

On the other hand, when the depth of the liquid is very small, the destructive process is more rapid, and no turbidity is to be detected.

In those cultures which have been grown in bright sunlight the bacteria are not appreciably diminished in virulence, so that, viewing the matter of disinfection by sunlight in the light of these facts, it appears that too much reliance cannot be placed in the bactericidal action of the solar rays, even in tropical countries.

Whether it is safe to argue from these experiments that the same results follow when pools containing nitrogenous matter are infected with cholera and warmed by the sun, cannot be rashly decided. It must be remembered that in one case one is dealing not only with cholera, but with many other forms of bacterial life, while in these experiments pure cultures have been employed.

It is, however, interesting to know, when speaking of sunlight as a germicidal agent, that it is not enough to regard exposure of cultures to the sun as sufficient for the complete destruction of bacteria. It must be remembered that to attain this end it is necessary that the exposure should be in the presence of air, and, moreover, that the contact must be free; as otherwise, instead of destruction, growth may occur, and the very thing brought about which it is desired to avoid.

ATHEROMA.

(II)

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Sussex County Hospital.*

(PLATE XVIII.)

THE TOPOGRAPHICAL DISTRIBUTION OF AORTIC ATHEROMA AND ITS CLINICAL SIGNIFICANCE.

THE frequency with which atheroma attacks aortic ostium as a delicate elevated tracery has been alluded to elsewhere¹ I propose in the following pages to give the results of some further observations on the earlier phases of linear atheroma, with special regard to its topographical distribution along the aorta. For this purpose it will be convenient to consider the artery as divisible into sections. The first of these extends from the ridge forming the uppermost limit of the Valsalvan sinuses to the orifice of the innominate artery,² the second includes the remainder of the arch, whilst the third section comprises the rest of the artery.

The circumference of the aortic ostium, along the ridge of the Valsalvan sinuses above mentioned, is divided by the orifices of the coronary arteries into two unequal arcs; that including the attachments of the right posterior flap being as a rule somewhat the longer of the two.³ The shorter and, for our present purpose, the more important arc, passes through the point of joint insertion of the free edges of the anterior and the left posterior aortic flaps. It also includes that portion of the intima whence springs the band of supplementary fibres which assist in forming the floor of the aortic arch. These fibres have been fully described in a former article.⁴ As they have not been mentioned elsewhere to my knowledge, and as they undoubtedly strengthen the aortic walls at the commencement of the artery, just where additional strength is presumably necessary, I feel justified in bringing them again before the

¹ *Journ. Path. and Bacteriol.*, Edin. and London, vol. iii. p. 9.

² Atheroma of the sinuses themselves has been already discussed.

³ The positions of the coronary orifices in relation to the point of joint attachment of the free edges of the anterior and left posterior aortic flaps vary considerably in different subjects. They are, however, mostly as above described.

⁴ *Loc. cit.*, pp. 5, 6.

notice of histologists. In the present instance the chief interest consists in their relationship to the linear growth of atheroma along the floor of the aortic arch.

At the commencement of the arch the earliest indications of atheroma are usually met with in the intima, immediately above the shorter of the two arcs, into which the circumference of the aortic ostium may be divided. In young subjects, and possibly in all, who have succumbed to a rapidly fatal disease, the preference of atheroma, if it exists, for certain sites is usually demonstrable. In the more chronic forms of the disease this peculiarity is frequently masked by secondary deposits elsewhere. Even in the latter case, however, the pathologist can often infer the locale of an early outbreak of the disease by the position of an ulcer, or from the presence of a calcified plaque amidst plaques, it may be, of linear atheroma (Plate XVIII. Fig. 6).

To a superficial observer there are few, if any, points of resemblance in the designs traced on the aorta by atheroma. If, however, we compare a series of specimens of aortic ostia, attacked by acute linear atheroma, we can scarcely fail to note one similarity in all the cases, and that is the shape of the atheromatous streaks or lines. These streaks, which are well shown in the two illustrations of thoracic aortæ (Plate XVIII. Figs. 7, 8), are found occasionally throughout the arterial system, and associated with a great variety of diseases. They have also a peculiarity in common, no matter what their situation,—a peculiarity which I find did not escape the observation of the late Dr. Moxon,¹—and that is, the majority lie with their long diameters parallel to the direction of the blood stream. At the aortic ostium linear atheroma not unfrequently assumes the form of whorls, rings, and sinuous lines; in the descending thoracic aorta, however, and in the lower abdominal region, it is usually to be seen in its simplest forms, as dots, or as narrow straight elevated streaks, about a tenth of an inch in breadth, and of a variable length, some measuring an inch or more. I shall, therefore, begin this account of the topographical distribution of linear atheroma by describing what may be seen when it affects the descending aorta.

The fixed position of the descending thoracic aorta along the spine, its directness, its tubular shape, and its freedom from large branches, together make it the most suitable and interesting vessel to study in the present inquiry. For it is here that we may assume the blood to move onwards in an unchecked "viscous flow," if this motion occurs anywhere in the aortic system.² It is here, then, that we may expect to observe the deposition of aortic atheroma under the simplest physical conditions; and, needless to add, under those most favourable to the present investigations. Two cases, recently in the Sussex

¹ "Atheroma forms lines lengthwise to the vessel's course."—Moxon, 1871.

² We may, at all events, assume that the blood flows viscously through this vessel, when the circulation is sluggish by failure of the cardiac propulsive power.

County Hospital, are typical examples of linear or streaky atheroma in this region, and they will serve as illustrative texts upon which to append the following remarks. That similar cases are seldom described in post-mortem records is no conclusive proof of their pathological rarity, since the descending aorta is generally left unopened at these examinations. In neither of these autopsies did the condition of the cardiac valves and aortic ostium suggest the probability of extensive atheroma of the descending aorta. The illustrations (Plate XVIII. Figs. 7, 8) are from photographs of the aortæ in question, taken from absolutely untouched negatives.

The first illustration (Fig. 7) represents the aorta of a man who died with symptoms of "rheumatism," associated with hyperpyrexia. The history of the case is given elsewhere,¹ and it is therefore unnecessary to recapitulate. The peculiarity of the aorta consisted in its apparent freedom from disease, until the descending part of the arch was reached, when the intima became flecked with streaks of atheroma, like those above described. At first, that is at the head of the descending aorta, the streaks were distributed generally over the intima. Even here, however, the streaks mainly had their long diameters either parallel to the general direction of the blood stream, or with a marked trend thereto. This peculiarity of disposition is in accordance with the behaviour of atheroma in other parts. When the level of the second pair of intercostal arteries was reached, although many streaks still flecked the intima on either side of their orifices, yet along the dorsal aspect of the vessel, in the intima between the intercostals, the lines of atheroma were more closely crowded than elsewhere. At a still lower level, this concentration of the disease to the site above mentioned was very evident, few if any streaks passing beyond the lines uniting the openings of the right and the left intercostal arteries respectively. Unfortunately, we had not an opportunity of examining the artery beyond the diaphragm, and the topographical description of the diseased vessel must end here. Coincidentally with the above peculiar disposition of *acute* atheroma,² the whole endothelium over the affected site was extremely friable and readily detachable from the outer coats of the intima, especially over the elevated streaks. Many of these presented rough, eroded surfaces. The inmost layers of the artery at these spots were seen under the microscope to be "frayed out," as it were, and crowded with "nuclear bodies," or vagrant leucocytes, few of which appeared in the deeper layers of the intima or media. There were, however, some swarms of highly-stained nuclear bodies scattered through the loose areolar tissue immediately surrounding the vessel.

¹ Appendix A, No. 66, p. 379.

² I think the history of the case justifies this appellation. The condition of the arterial intima tallied fairly well in its general details with the description of acute arteritis given by Rokitsansky (Bibliography).

In the other specimen of linear atheroma of the descending aorta,¹ from a case of uræmia (Plate XVIII. Fig. 8), the streaks were few in number throughout the first two-thirds of the arch. It was not until the descending aorta was reached that the disease attained its full development. From this point downwards the inner membrane of the artery was furrowed with numerous short, beaded ridges. Especially was this the case on that strip of intima between the orifices of the intercostal arteries, which, we may remember, proved to be peculiarly susceptible to the attacks of the disease in the former instance. The atheroma was not, however, limited so strictly to this region, as it apparently was in the more acute attack of hyperpyrexia. Commencing at the left-hand corner of the figure, and passing obliquely downwards towards one of the lower intercostals is a light-coloured band, which represents a broad elevated streak of atheroma. It also represents with considerable accuracy a line joining the termination of the floor of the arch and the dorsal aspect of the thoracic aorta, that is, two sites much affected by atheroma in its early stages. The surface of each atheromatous streak was smooth and rounded, and the intima was everywhere firmly adherent, in marked contrast to the other specimen. A section of one of the atheromatous streaks was stained and otherwise prepared for microscopic examination, in a manner as far as possible identical with that employed in the other case. It was then found that the second specimen differed in many important details from the other section, which I have above described. First, a few scattered swarms of nuclear bodies alone had invaded the endothelium at rare intervals, leaving wide reaches of untouched membrane, and they presented an appearance very different from the more general invasion of the endothelium observable in the former example. Very different, too, was the behaviour of the individual nuclear bodies in the two subjects. The specimen we are now considering was riddled, as far outwards as the middle coats with the minute canals, or "burrows" as I prefer to regard them, associated with the dissemination of nuclear bodies throughout the elastic layers of the aortic walls. Each burrow, as a rule, contained one, rarely more, of these deeply stained, pip-shaped bodies. In some cases these bodies seemed to be encapsuled among the fibres of the walls. Others from their faint colour, larger size, and more granular texture, I judged to be undergoing a process of dissolution, such as I described in my previous paper. In the case of hyperpyrexia, it will be remembered, none of these nuclear bodies seemed to penetrate outwards beyond the innermost layers of the intima which were torn and frayed out by the number and the violence of the invaders. Few, if any, were in that case encapsuled, but of those which had penetrated most deeply, many were probably undergoing dissolution.

There is in the histories of several of the cases recorded in the

¹ Appendix, Case No. 59, p. 377.

appended table indirect evidence pointing to the occasional formation of an atheromatous streak within a few days of death. For instance, in the case of a healthy child of 4 years of age, who died 4 days after severe burns, the aorta was streaked with atheroma (Plate XVIII. Fig. 1). In the former paper two or three cases were cited, in which the historical testimony favoured the view that the aortic disease commenced within 10 days of death. More recent evidence on this subject, however, makes it probable that atheromatous tracery may be produced upon a healthy intima within a week. Now let us consider in what way the rapid production of atheroma under certain conditions can tell upon its distribution along the aorta. If we assume, for example, that many streaks of atheroma visible in the aorta of the man, who died of hyperpyrexia, were the growth of a week or less, and neither their aspect nor the history of the case is opposed to this view, we tacitly admit that the arterial mischief was in active progress when the patient was lying supine and helpless in bed; when his dilated heart strove vainly to compensate by rapidity of contraction for the gradual failure of its propulsive powers. Now, if foreign particles of a higher specific gravity than plasma were carried along the aorta by a sluggish blood stream, whilst a patient was lying on his back in bed, they would tend to occupy the lowest strata of the liquid plasma. The location of linear atheroma along the dorsal aspect of the thoracic aorta tallies well with this interpretation of its causation by such foreign particles. We have, however, a still more cogent argument in favour of the hypothesis that, in both the cases I have described above, this arterial disease was due to the introduction of heavy foreign particles into the blood.

Leucocytes, as is well known, are specifically lighter than blood plasma. During their life, however, the want of density does not apparently inconvenience these minute bodies in their progress through the vessels. Nevertheless, this physical relationship between leucocytes and blood plasma must be always present in the living body, always ready to make its influence felt when the time and the place are favourable to that end. There is constantly a tendency, in accordance with the law of gravity, for the blood to differentiate itself into horizontal layers, the heavier particles sinking to the lower strata, and the lighter floating upon the top. During health, when the organs of our bodies are functionally active and vigorous, the operation of this law is unnoticeable. It is, however, otherwise, if through the failure of the heart's contractile powers the blood flows sluggishly within the great nutrient vessel. Then we may expect specific differences of weight among the contents of the aorta to show themselves the more readily, as the circulation slows. When a patient has been lying for some days or weeks upon his back in bed, as in the two cases I have just recorded, we should be justified in assuming, if any change took place in its constitution, that a hyperleucocytosis existed in the upper or

ventral layers of the aortic blood, and that in the dorsal or lower strata there would necessarily be, during the last days of life, a hypo-leucocytosis. Now what did the *sectio cadaveris* teach us in regard to this conjecture? Why, that it was quite wide of the mark. All the pathological evidence at our disposal showed, as plainly as such evidence can, that the contrary condition existed in the blood of the aorta at the time of death. That in the first case, especially, the lowermost layer of the blood must have then been swarming with leucocytes; leucocytes which for some set purpose, if such creatures can have purposive actions, had, despite their specific lightness, forced their way through the blood plasma to the dorsal aspect of the vessel. I know of only one explanation of this phenomenon, but that is happily a simple one. The leucocytes visible in the thin sections I have described, as adherent to the walls, or as passing into the layers of aortic intima, were phagocytes, which, after a heavy meal, strove, as is their wont, to free the blood from the contamination of themselves and their ingesta by the shortest possible route.¹ The above facts all tend to confirm the view that the presence of linear atheroma, along the dorsal aspect of the descending aorta, is due to the admixture of specifically heavy foreign particles with the blood.

As regards atheroma of the first section of the aorta, that immediately above the valve, we have here to deal with a tube which is structurally more complex than the one we have just been considering. Owing, also, to the nearness of the heart, its contents are doubtless subjected to greater jerks and jars, to more violent waves and eddies than those which affect the blood elsewhere. Under these physical conditions we cannot expect linear atheroma, if the blood current in any way participates in its formation, here to assume the comparatively simple aspect that it does in the descending aorta. As a matter of fact we find, as I have already stated, that the lines or streaks of incipient atheroma are frequently twisted and otherwise distorted at the commencement of the arch. The loops, whorls, and rings, so commonly seen in this situation, are comparatively rare in the straight descending artery (cf. Plate XVIII. Figs. 5 and 6). There is, however, one topographical peculiarity, affecting both the straight and the sinuous lines in the former position. The disease has a tendency to spread along the floor of the arch, much in the same way, apparently, as it spreads along the dorsal aspect of the thoracic aorta. Not only is this the case, but it frequently happens that other streaks situated around the ostium, yet away from the floor, have a distinct trend on either side towards that part; that is to say, every such streak would form, if produced, a more or less acute angle,

¹ This subject has already been somewhat fully considered (*loc. cit.*, vol. iii. p. 15). It is probably outside the present inquiry to speculate how leucocytes can sink in the blood, in opposition to their gravity, and I do not feel inclined to act as nature's apologist in this matter. I may suggest, however, that by closely adhering to the endothelium, leucocytes possibly move without difficulty in many directions along a vessel's walls.

with a line passing longitudinally along the centre of the floor. I have measured, in different cases of well-marked trending, the angles so formed on either side of an imaginary base line along the floor of the arch. I shall not reproduce these measurements here, as they were necessarily approximate only, and until they can be more accurately determined it is needless to publish them. I may state, however, as a general result of several observations on seven different subjects, in five of them streaks near the left attachment of the anterior cusp that formed angles considerably over 45 degrees (but less than 90 degrees) with the line in question, while others adjacent to the right attachment of the right posterior cusp formed, when produced in the manner suggested, with one exception only, angles very much smaller than 45 degrees. It will seem, then, that in these cases there is, first, apparently a tendency for streaks of atheroma to pass along the floor of the aorta, with their long diameters in the general direction of the blood stream. Secondly, that streaks on either side of the floor, yet not actually upon it, frequently have a trend in the same direction.¹ In chronic forms of atheroma these peculiarities of its growth along the first section of the arch are usually masked, and sometimes obliterated.²

As regards the remainder of the arch the chief interest centres in the terminal portion, just above the descending thoracic aorta. At its union with the latter the arch appears to twist somewhat upon itself. Just below the orifice of the left subclavian artery, and upon the opposite side of the arch, is the real termination of the floor. This spot, where the intima is somewhat uneven owing to the presence of bundles of supplementary fibres, is frequently the seat of atheroma in its early stages (Plate XVIII. Fig. 2). The openings of the large vessels are also often affected, although in these cases atheroma spreads mostly along the sides of the branch vessel, and not at first along the aortic walls. There is usually at the head of the descending thoracic aorta a slight dilatation anteriorly. This is often splashed with atheroma; indeed, the arrangement of linear atheroma at this part of the aorta is very irregular, and there is apparently an absence of that preference for particular sites so noticeable in other divisions of the artery.

The following conclusions are the outcome of the observations discussed in the foregoing pages:—

1. There are satisfactory clinical, pathological, and histological evidences that atheromatous streaks may be formed during the last few days of life by the passage of leucocytes more or less deeply into the lining membranes of a previously healthy aorta.

¹ As the imaginary base line along the centre of the floor of the aortic arch is difficult to determine with accuracy, it has been found advantageous in practice to use as base lines, first, a line passing through the uppermost limits of the attachments of the anterior cusp; secondly, a similar line through those of the right posterior cusp. These base lines are far more satisfactory than the other, and show the amount of trend of an atheromatous streak in this locality with facility.

² I have elsewhere considered how these changes arise (*loc. cit.*, p. 10).

2. The presence of linear atheroma, specially along the dorsal aspect of the descending aorta, in subjects who have spent the last few days of life upon their back in bed, suggests that the leucocytes, which are specifically lighter than blood plasma, were attracted to that side of the artery in opposition to the law of gravitation.

3. In these cases—(1) the previous introduction of heavy foreign particles into the blood; (2) the gradual subsidence of these particles; (3) their subsequent ingestion by phagocytes; and (4) their rapid removal by these bodies from the circulation, together offer a simple explanation of the pathological phenomena.

4. The favourite situation of linear atheroma of the aortic arch is the floor or lesser curvature.¹

5. The atheromatous streaks usually point with their long diameters in the direction of the blood stream.

SECTIONAL CALCIFICATION OF THE AORTA, FROM AN ANATOMICO-PATHOLOGICAL STANDPOINT.

In the former article on atheroma a considerable amount of evidence was offered in favour of the hypothesis that the arterial disease was always preceded by the introduction into the blood of minute foreign particles, injurious to the host. The observations recorded in the earlier pages of the present paper confirm, in an indirect manner, the above proposition as regards linear atheroma, a disease peculiar to childhood, youth, and early middle age. They, however, leave untouched the etiological relationship of that rare and interesting disease of advanced life, wherein the aorta along a part of its length only is more or less completely calcified. When the area of intima affected in this extreme manner is limited to the abdominal aorta, the remainder of the vessel being relatively free from the disease, as happened to two patients formerly under my care,² it would seem probable that some supplementary factor of importance in the causation of atheroma had been omitted from consideration. It was to ascertain to what extent this peculiar disposition of calcareous atheroma affected the validity of what had heretofore seemed a good working hypothesis, that I undertook the following inquiry.

I have, unfortunately, been unable to collect more than six cases of extreme calcareous atheroma of the aorta, notes of which are given in the appended table. Four of the cases have come under my personal observation, and of the remainder, one is the hospital specimen above mentioned (No. 4); the other consists of a case recorded by my

¹ The situation of acute atheroma along the floor of the arch by preference may account for the rarity with which this disease of the intima terminates in aneurism, which usually springs from the convex side (Bibliogr. *Oppolzer, Gee*). It is very doubtful whether atheroma is often the cause of aneurism (Thorburn).

² Case No. 5, vol. iii. p. 23, and Case No. 53, p. 376.

colleague, Mr. T. J. Verrall (No. 55), and photographed by Dr. A. J. Richardson. Of the whole number two only are examples of sectional calcification of the abdominal aorta (Nos. 5 and 53). We must not, however, conclude that this disease is consequently exceptionally rare, but rather that, from one reason or another, it has not attracted the attention of pathologists, and the conservative energy of curators of hospital museums.¹

If we separate the alimentary canal into two hypothetical divisions at the pylorus, we shall obtain two tracts of mucous membrane, differing largely in function. The upper portion consists mainly of a tube for the reception and preparation of the crude materials from which our bodies derive their health and strength. On the other hand, within the intestinal canal is situated the most important absorbent system we possess, for here the prepared food is absorbed, and to some extent even incorporated within us. This arrangement of the alimentary system lessens the risks we all run of direct blood contamination, through the introduction of injurious foreign particles with the chyle. For owing to the distance the pylorus is from the mouth the chance of aërial infection is diminished, and, in submitting the food to the processes of mastication and stomachic digestion, it is mainly rendered aseptic, and many living germs introduced along with it are probably destroyed, that is to say, so long as the organs are functionally sound, and the epithelial lining intact.² The nutrient vessels of the alimentary canal throughout the greater part of its length are, as we may remember, somewhat peculiar. They are noted for the large number of arterial anastomoses between the branches of the chief nutrient vessels. This is specially the case as regards the lower section of the canal; and a reason for the arrangement here is probably an obvious one. It must undoubtedly be important that the nutrition of such a functionally active absorbent system should be always efficient, even under occasionally adverse conditions. Yet the intestines deal largely with crude food material as it passes the pylorus, and render this stuff both chemically and physiologically capable of healthful assimilation within our bodies. In dealing with this material, I imagine, there must be an occasional, I shall not call it a frequent, risk of the temporary blockage of some of the terminal arterioles, by the accidental introduction of crude particles into the

¹ As far as I am able to ascertain, the specimen in the Museum of the Sussex County Hospital is unique; at all events, it is doubtful whether the metropolis can furnish a similar specimen. Unfortunately, as is so often the case with ancient museum specimens, the clinical history is deficient. Guy's Hospital, I believe, possesses one of the finest collections of diseased aortæ in England; but even here some of the older specimens are deficient in this respect.

² If the researches of Kaufmann are correct, different micro-organisms behave differently in the presence of free hydrochloric acid; generally speaking, those which split up the carbohydrates, and those causing lactic acid fermentation, are most resistant. On the other hand, those that split up nitrogenous material are the most susceptible.

blood stream. Now the system of arterial anastomosis mentioned above will reduce the inconvenience, not to say danger, of an arterial block to the nutrition of the mucous membrane surrounding it. The circulation in such a patch of membrane will, despite the thrombus, be carried on much as usual, until phagocytes have removed the impediment. It is conceivable that a small artery may be obstructed in various ways, for instance by fat globules, or by the introduction even, through osmosis, of some free acid or other reagent having the property of chemically coagulating the blood, and not necessarily by an invasion of living microbes. The risk of accidental blockage from one of the above causes must be incurred, if at all, by the mesenteric arterioles, and I think that this explanation of the necessity for arterial anastomoses in this circulatory district is a plausible one. It may not be the only reason for the peculiar vascular arrangement we here find, possibly it is only one of many.

When, however, we turn our attention to the upper division of the mucous tract, that above the pylorus, the anastomotic character of the arterial circulation cannot be accounted for in the above manner, for although the mucous membrane of the stomach may have some absorptive power, the pharynx and œsophagus can scarcely have functions of this kind. Yet it is especially with this part of the alimentary tract that I am about to deal. There is, moreover, a further vascular peculiarity as regards the lower part of the œsophagus and the cardiac end of the stomach, one set of their anastomotic vessels arises as small short branches directly from the aorta. The arteries supplying the remainder of the tube, *e.g.* the ascending pharyngeal, the superior thyroid, and the inferior thyroid, anastomose freely with each other, and thereby with the upper œsophageal branches of the aorta, although their relations with the largest artery of the body are not so close, it may be, as are those of others lower down. We have, at all events, one district of mucous membrane in very near circulatory relationship with the aorta, namely, that of the lower gullet and stomach cardia.

Although leucocytes are endowed with certain spontaneous locomotory powers, it is improbable that they can make headway against the blood current even in the capillaries, where the flow is sluggish. In the aorta and large arteries, where the flow is rapid and often turbulent, the majority are swept onward with the blood. It is probable, too, that the greater number of bacteria, when introduced into the vessels, are also carried along by the blood, although they may be capable of independent locomotion. Intermittent and desultory movements in particles suspended within a liquid stream will not, I take it, greatly affect their translation by the stream through space. When, however, an artery is blocked at its distal end, and the circulation through it has ceased, there exists no impediment to the free movements of any particles capable of locomotion in the contained

blood so long as the latter remains fluid¹ on the proximal side of the thrombus between it and the next arterial orifice. We may expect, under these conditions, that both leucocytes and bacteria may occasionally pass backwards through the stagnant blood to the nearest vascular opening, to be again swept onwards to the capillaries by the blood stream.

Arterial anastomosis favours the blocking of an artery at the distal end, when a thrombosis takes place within an anastomotic district; for, of two inosculating vessels, one, usually the smaller, must necessarily be obstructed at its distal end, unless the whole vessel is occluded by the clot, an occasional accident. In the former, and I imagine the more usual case, an arterial *cul-de-sac*, blocked at one end by a clot and containing fluid blood, will be the result. This vascular recess, if the thrombus was due to infection, might speedily become the breeding-place of pathogenic microbes, and the happy hunting-ground of leucocytes. Hence, engorged phagocytes and bacterial swarms might readily find access to the general circulation.

Let us now return to the six cases of aortic calcification, which were alluded to at the beginning of this article (Nos. 4, 5, 20, 53, 54, and 55 of Table). The selection of these cases has mainly depended upon the following circumstances. Of three (*viz.* Nos. 20, 53, and 54) I still possess portions of the aortæ. One specimen is in the Sussex County Hospital Museum, as above stated; and of one other I have the photograph, as before mentioned. One alone of six cases rests its claims to inclusion with them upon a historical description. As, however, the description was written by myself shortly after the autopsy, or upwards of a quarter of a century before this paper, and many years before I had turned my attention to the investigation of atheroma, personal equation cannot, I imagine, have prejudicially affected it. As regards the disposition of the atheroma, the disease affected the arch, the thoracic, and the abdominal aorta in a third of the cases respectively. Of the two in which the arch was attacked, one had kidney fibrosis and hæmorrhagic ulcers of the hard palate, and the other had a somewhat doubtful history of a malignant gastric ulcer. The calcification of the thoracic aorta was associated with malignant hepatic disease in one case, with malignant disease of the œsophagus in the second. Finally, there was a definite history of extensive and chronic ulceration of the stomach in both the cases of calcification of the abdominal aorta. The ulcers had invaded the cardiac extremity of the organ in each instance. We have then in these six cases of extreme calcareous changes in the aortic coats, a history of extensive ulceration of the upper part of the alimentary canal in five, and of malignant disease of the liver in one. Again, there is a clear history

¹ It is well known that stagnant blood may remain fluid within a living vessel for a long period; the experiments of Lister with the jugular vein of a horse proved even more than this (*Bibliogr.*).

of malignancy, in the sense usually ascribed to this word, in three patients; and in two others the disease of the stomach was probably of this nature. In patient (No. 20) with fibroid disease of the kidneys there was no evidence whatever pointing to a malignant taint. Malignancy seems, as far as my experience avails me, to play an important, although by no means essential, part in the causation of aortic calcification.¹

It is, however, to the association of aortic calcification with one or more ulcers in the upper section of the mucous tract that I wish to direct attention at the present time. In all the six cases, save one, there is a history of chronic ulceration—mostly of a hæmorrhagic type—in some part of the alimentary canal above the pylorus. In the excepted case, one of malignant hepatic disease, the history is so imperfect, and the possibility of the malignant growth being of a secondary nature so considerable, that I shall omit it from consideration at present. There remain five cases of chronic hæmorrhagic ulcers of the mucosa. As the interest of these cases centres mainly in the sectional calcification of the abdominal aorta, I shall commence with a consideration of the two cases (Nos. 5 and 53). In each of these subjects the ulceration was confined to the cardiac end of the stomach. The first was undoubtedly carcinomatous. The whole of the cardia was occupied by a large ragged ulcer, about 4 in. across, and nearly circular in shape. The walls of the stomach outside the ulcers were firmly adherent to the adjacent viscera. Many secondary growths, circular in shape and with depressed centres, were visible beneath the peritoneal coat of the liver, others were found in the lungs, vascular, yellowish tumours, in some instances with gritty or caseous centres. Under the microscope, the growth had the histological characters of carcinoma. The abdominal aorta was converted into a rigid calcareous tube for about 4 in. above the bifurcation. There was atheroma elsewhere, at the aortic ostium and at the commencement of the arch, but the disease was comparatively slight. The thoracic aorta was dilated. In the second case, on the posterior surface, and close to the cardiac orifice at the commencement of the lesser curvature, were two ulcers, with pigmented surfaces and somewhat thickened submucous bases. The larger had an area of $1\frac{1}{2}$ in. by $\frac{1}{2}$ in. A tortuous vessel passed close to the ulcers. The intestines contained much blood clot. There had been repeated hæmorrhages from the bowel during life. There were no secondary growths in any viscus. In this case, as in the preceding one, atheroma had attacked

¹ A search through the Metropolitan Museums for cases in any way resembling those above alluded to has been for the most part fruitless. In Guy's Museum the catalogue refers to a specimen (1462), "a portion of aorta with spots of earthy deposit, from a man who died of cancer," but the specimen had apparently been removed. In the University College Museum (3047), there is a specimen of pyloric ulceration, associated with which was a small aortic aneurism with calcareous walls.

the cardiac valves somewhat, and a few patches of the disease were seen on the thoracic aorta, which was also dilated. It was not, however, until the diaphragm was passed that the full extent of the vascular mischief was obvious. The aorta had apparently been converted into an inelastic calcareous tube. In these two cases, then, we have two remarkable coincidences, namely, extensive ulceration of the mucous membrane of the cardia, associated with those rare changes in the coats of abdominal aorta, which I have named *sectional calcification*. The coincidences are rendered the more interesting when we remember the close vascular connection there is between the cardiac end of the stomach and the aorta, through the arterial anastomoses of the coronary artery of the stomach with four or five aortic oesophageal branches. Any large and deep excavations in the stomach walls, situated as these ulcers were at the cardiac orifice, would necessarily interfere with the anastomotic vessels, blocking some and destroying others. It is conceivable, owing to its shortness and its direct relationship with each affected district, namely, the cardia on the one hand and the aorta on the other, that one or more of the chief oesophageal branches might be occluded by a clot, and a direct backward communication established with the abdominal aorta in the manner I have described. If the deposition of calcareous matter within an arterial wall is brought about by the agency of leucocytes, by a method analogous to that described elsewhere,¹ the sudden incursion of a horde of half-poisoned phagocytes into the aorta at the spot mentioned would probably lead to a sectional calcification of the aortic walls, similar to that actually found in Cases 5 and 53, unless it produced a still more serious, because a more acute, condition of the main nutrient vessel of our bodies and its contents. Why sectional calcification of the abdominal aorta should be, under these circumstances, an unusual accompaniment of gastric ulcers depends doubtless on a variety of causes, some of which I shall now recapitulate.

An important factor in the etiology of sectional calcification of the aorta, if the oesophageal arteries enact the part I have assigned to them, will be the position of the stomachal ulcer.² It is evident that unless the ulcer is so situated at the cardiac orifice as to interfere with the flow of blood through one of the main oesophageal branches of the aorta, the sequence of pathological events, suggested in the case of abdominal aortic calcification, cannot take place. Conversely we might

¹ *Loc. cit.*, pp. 11, 12.

² Drs. Perry and Shaw, in their careful analysis of the specimens of malignant disease of the stomach in Guy's Hospital Museum, found the pylorus involved in 70 per cent. of the cases. The lesser curvature rarely escaped. The greater frequency with which the pylorus is attacked in these cases may help to account for the rarity of the associated aortic disease. Of perforating gastric ulcers, Mr. Barling says: "A large majority of these ulcers occur on the posterior surface and lesser curvature. A few occupy the region of the pylorus, and a still smaller number involve the anterior surface of the stomach."—"Ingleby Lectures," 1895, iii.

expect sectional calcification of the thoracic aorta, as distinguished from that of the abdomen, to be associated, if at all, with ulcers of the alimentary canal, situated at a higher point than the stomach. I have unfortunately been unable to obtain more than one example of extreme atheroma of the descending thoracic aorta with a sufficient clinical history. It is Mr. Verrall's case of a carcinomatous ulcer of the œsophagus, perforating the trachea (No. 55). There is no mention of the exact position of the ulcer in this case, but from the fact that it perforated the trachea it must have been situated several inches above the stomach. Now in this case the descending thoracic aorta was the vessel most seriously affected, and the orifice of the uppermost œsophageal artery was apparently embedded in a thick atheromatous plaque, which plainly showed that the aortic disease extended so high in the thorax (Plate XVIII. Fig. 9). The inferior thyroid artery anastomoses with this aortic branch. Such evidence as this single case affords favours the assumption that sectional calcification of the descending aorta depends largely upon the anatomical arrangement of the nutrient arteries of the œsophagus and stomach. Although I have been unable to obtain any other case of sectional calcification of the thoracic aorta, with a satisfactory clinical history, I have met with a case of fungoid aortic growths, which will throw some light upon the subject we are considering.

The small branches given off by aortæ just below the arch are irregular in their origin. There is in a certain percentage of subjects a small œsophageal branch, which arises close to the termination of the floor of the arch, about half an inch below, and on the opposite side to the opening of the left subclavian artery. This branch, when it exists, is considerably above, and not in a line with, the highest right intercostal artery. It is also much smaller than this vessel. I imagine that the former becomes not seldom blocked and obliterated. At all events, I have met with a minute depression on the intima at the spot where the uppermost œsophageal branch usually comes off, in subjects where this artery was apparently absent.

On 11th February 1893 an old man was admitted in an emaciated and exhausted condition to the Sussex County Hospital, where he died two days afterwards. There was a history of progressive emaciation, associated with dysphagia for two years previously. On opening the œsophagus a patch of ulceration, about an inch in length, and reaching almost around the tube, was found on a level with the bifurcation of the bronchi. A few fungoid-looking growths were attached to the wall of the œsophagus. There was very little thickening. A slit-like opening, about half an inch long, was visible between the base of the ulcer and the trachea. The opening was just over the right bronchus. The arch of the aorta and the apex of the left lung were adherent to the growth. Several cavities filled with pus, and with smooth walls, were found in the upper lobes of lungs; the largest at the right apex. On cutting into the aorta, the lower wall of the arch, which was adherent to the growth, presented a fungoid-looking vegetation, soft, and readily peeling from the intima, and about the size of a threepenny-bit. The adventitia at the base of

this growth was apparently healthy. There were two or three smaller patches, presenting a similar appearance, lower down. The heart was healthy.¹ On examining the aortic growth and the subjacent patch under the microscope, the former consisted apparently of a fibrous stroma, with a loose irregular network, within the meshes of which were many "nuclear bodies," or vagrant phagocytes. The patch was formed of the aortic intima, and possibly the inner layer of the media, which had been invaded by leucocytes, and, as usual, frayed out and damaged by them.

We have here an example of a soft fleshy growth upon a patch of atheromatous tissue such as is commonly found upon the cardiac valves, and rarely elsewhere. Now the chief interest in this case arises from the situation of the aortic growth, and its relations to the œsophageal ulcer, which, by the way, was on a squamous epithelial carcinoma. The site of the aortic patch was adjacent to the orifice of the uppermost œsophageal artery, the chief nutrient vessel, with the inferior thyroid of that part of the mucous membrane destroyed by the ulcer. Unfortunately the condition of the artery was not observed at the autopsy, and I am unable to state whether it was blocked by clot or not. However that may be, it is scarcely credible that a vessel connecting the ulcerated œsophageal surface with the diseased aortic intima would come off scathless. Again, the extremely local character of the aortic mischief pointed to some equally local cause at work producing it. If a plug was formed in the upper œsophageal branch of the aorta, a line of communication between the mucous ulcer and the aorta might at any moment be established through the stagnant blood, in the way I have previously explained. That the aortic intima immediately surrounding the vessel's orifice became diseased under these circumstances would not be surprising, for this case tends to show, like those I have before alluded to, that ulcers of the gullet may, through the arrangement of the œsophageal nutrient vessels, determine the position of an atheromatous aortic plaque.²

Other factors in the causation of sectional aortic calcification besides the *position* of a chronic mucous ulcer are doubtless its size and depth, the shape and condition of its walls, and so forth. It will thus be seen that the exceptional rarity of sectional calcification in the abdominal portion of the vessel is not surprising, especially when we consider the frequency with which an examination of the aorta is omitted at autopsies.

Of the three remaining cases of aortic calcification, not yet referred to, the arch was the part chiefly affected in two. In only one of these subjects, a patient of mine, was the history quite satisfactory. This

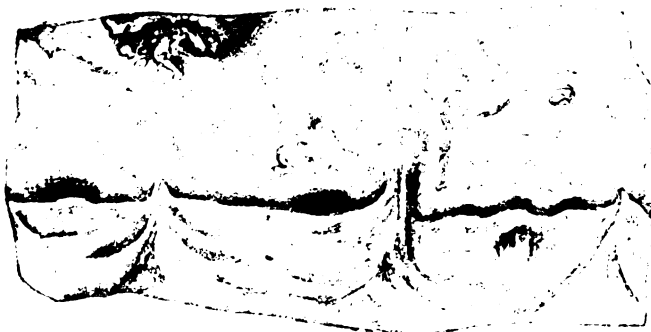
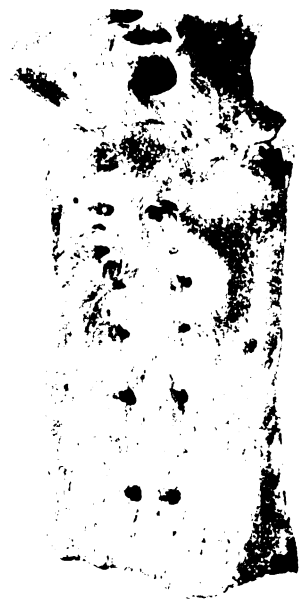
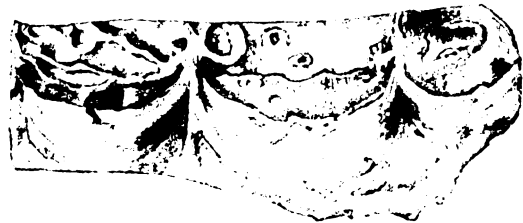
¹ I am indebted to Mr. Mallam, a former house physician, for the notes of the above autopsy (No. 69).

² In the museum at Guy's Hospital there is a specimen (No. 1501²⁴), described as a "small blood clot, the size of a filbert, adherent to a slightly atheromatous patch on the aorta, just above the pillars of the diaphragm. Patient, 66, admitted with epithelioma of the œsophagus in a very emaciated condition." This case doubtless resembled the one I have above quoted in many particulars.

case was one of extensive atheroma, along with numerous ulcers in various parts of the arch and at the commencement of the thoracic aorta. The patient, a stableman, suffered from renal inadequacy,¹ and, towards the end of life, from frequent hæmorrhages from the buccal and intestinal mucous membranes. That from the mouth was found to come mainly from an ulcer situated on the roof, at the line of junction of the hard and soft palates. The nutrition of the mucous membrane of the pharynx and mouth, although largely carried on by anastomotic vessels, is not so closely connected with the aorta as is that of the lower gullet. Any infective material absorbed by an ulcer within this region would have to pass the usual circulatory round, in order to arrive at the aortic ostium. The length of the systemic series of vessels in such cases would be comparatively a short one to traverse, and it would be one that was remote from the chief blood purifiers, namely, the spleen and the kidneys. Under these conditions a larger percentage of the poisonous matter might pass the aortic ostium in a given time than would be the case if the foreign matter had found its way into the blood current at some more distant point. This, however, is only a question of degree, and not of kind. We cannot expect to find any special local peculiarities, differentiating atheroma of the aortic ostium, originating in chronic buccal ulceration, from that due to an iliac ulcer, for instance.

Finally, it may be contended that the above cases, although they show an association to exist between ulcers of the alimentary canal and atheroma, do not admit the interpretation I put upon them; and that the atheroma may have been the cause of the ulcer in many if not all the examples I have adduced. The old theory that gastric ulcers mostly originate "in affections of the vessels connected with the diseased area, especially embolism or degenerative change of the arteries," may be true to an extent. It is not, however, the whole truth. Degenerative changes in the arteries supplying mucous membrane, adjacent to an ulcerating patch, would probably lead to an enlargement of the area of the latter. Dr. S. Fenwick, who has studied the subject carefully, asserts that where the arteries are healthy in gastric ulcers, the veins of the mucous membrane are generally dilated and thickened. If we admit the occurrence of gastric ulcers, without the presence of diseased arteries, we undoubtedly greatly weaken the arguments of those who would make arterial atheroma the cause of all such ulcers. The cases narrated above show that a relationship exists between the position of chronic mucous ulcers of the upper part of the alimentary tract and the location of aortic atheroma. The only interpretation of the

¹ Dr. W. H. Dickinson called attention to the occurrence of ulceration of the bowel with granular kidney in the Croonian Lectures for 1876. More recently he has collected twenty-two examples. In two cases the stomach was ulcerated as well as the bowel. The most marked character of the ulcers was their association with hæmorrhage.—*Proc. Roy. Med. Chir. Soc.*, vol. vi. p. 36.



phenomena which satisfies both the anatomical and the pathological requirements of the case would seem to be the one that I have above given.

DESCRIPTION OF PLATE XVIII.

The plate contains figures of aortic ostia affected with atheroma. Three of them may be considered to represent fairly typical examples of the acute disease, whilst the others are examples of a more chronic form.

FIG. 1.—Atheroma of aorta after a burn, 4 days previously. Girl, 4½ years. Case 68.

FIG. 2.—Commencing atheromatous ulcer at the termination of the floor of the arch of aorta, after a burn. Boy, 3½ years. Case 67. (a) The ulcer.

FIG. 3.—Atheroma in a case of tubercle. Case 58.

FIG. 4.—Atheroma in a case of typhoid fever. Case 63.

FIG. 5.—Atheroma of the aorta from a case (No. 60) of granular kidneys, with symptoms of uræmia before death.

FIG. 6.—Extreme atheroma of the aortic ostium, from a case of malignant gastric ulcer. Case 54. (a) Atheromatous ulcer at the commencement of the floor of the arch.

FIG. 7.—Linear atheroma of the descending thoracic aorta, from a case of "rheumatic" hyperpyrexia. Case 66. From an untouched negative photograph.

FIG. 8.—Linear atheroma of the descending aorta, from a case (No. 59) of uræmia. From an untouched negative photograph. Figs 7 and 8 about half natural size.

FIG. 9.—Extensive calcareous atheroma of the thoracic aorta, in a case of carcinoma of the gullet. Case 55. From a photograph by Dr. A. J. Richardson.

APPENDIX.

Table of Cases of Atheroma, showing various Diseases with which the chief Organs were concurrently affected. Continued from p. 23.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys, etc.	Other Organs, History, Remarks, etc.
No. 53, M., 65, retired.	Heart, 26 oz.; tricuspid and mitral valves thickened; pulmonary ostium dilated; aortic valve atheromatous; some patchy atheroma over thoracic aorta; abdominal aorta almost completely calcified.	Right lung much collapsed; left, fibrous tissue thick; cut ends of vessels stood out rigid on section.	Capsules adherent; shrunken; renal arteries tortuous and atheromatous.	Close to the cardiac orifice of stomach, at posterior aspect of lesser curvature; two ulcers with pigmented thickened bases; large tortuous vessel passed near them.
No. 54, M., 50-60.	Aorta extremely atheromatous with much ulceration. Fig. 6.	Unrecorded.	Unrecorded.	A large (malignant?) ulcer of the stomach.
No. 55, M., middle-aged.	Descending thoracic aorta very atheromatous. Fig. 9.	Apex of left lung gangrenous. Mr. Verrall's case. ¹	Unrecorded.	Carcinomatous ulcer of the œsophagus, perforating trachea.
No. 56, M., 24, cabman.	Heart dilated; valves healthy; aorta atheromatous at commencement.	Pleural cavities contained fluid; right lung contained tubercles; left, collapsed and fibrous in parts; bronchioles dilated.	Congested.	Both layers of peritoneum studded over with tubercular granules.
No. 57, F., 13.	Edges of mitral curtain thickened; spots of atheroma on the aorta.	Pleura dotted with discrete yellow tubercles; lungs studded with grey miliary tubercles; bronchial glands tuberculous.	Bases of pyramids congested; a few yellow tubercles in cortex.	Pia mater contained many tubercular granules; bad cough 3 months; headache 10 days before admission.
No. 58, F., 23, wardmaid.	Heart, 7½ oz.; pericardium distended with serum; mitral valve atheromatous; also aorta. Fig. 3.	Right pleura adherent; lungs' weight, 44 oz.; cavities at both apices partly filled with reddish grumous fluid; recent tubercle at right base.	Weight, 8½ oz.; cortex plus and pale; no lardaceous changes.	Had suffered from winter coughs since childhood; frequent hæmoptysis; family consumptive.

¹ *Proceedings of the Brighton and Sussex Medico-Chirurgical Society, September 6, 1894.*

APPENDIX.—Cases of Atheroma—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys, etc.	Other Organs, History, Remarks, etc.
No. 59, M., 28, gardener.	Heart, 13 oz.; left ventricle hypertrophied; valves healthy; thoracic aorta atheromatous. Fig. 8.	Lungs intensely congested.	Weight, 5½ oz.; red and tough; cortex very thin; no cysts; urine, ½ albumen, scanty.	Symptoms of kidney mischief for 3 months before admission; semi-conscious, and with total suppression of urine 5 days before death.
No. 60, M., 32, fruiterer.	Heart, 20 oz.; cavities much dilated; aorta atheromatous. Fig. 5.	Some apical adhesions; lungs cedematous and congested; Fibroid changes at both apices, with scars on pleural surface.	Weight, 13 oz.; capsules thick and adherent; granular in parts.	Moribund when admitted; history of excessive beer drinking.
No. 61., F., 22, domestic.	Left ventricle hypertrophied; tough sprouting growth on mitral valve.	Pleural adhesions at apices; lungs congested.	Capsules adherent in parts; cortex mottled; weight, 19½ oz.	For history, see end of this table.
No. 62, M., 19, undertaker.	Heart, 23 oz.; right cavities dilated; mitral orifice contracted; flaps fringed with atheromatous sprouts; aortic valve also atheromatous.	Right pleura generally adherent; right lung cedematous; left, collapsed.	Right, 7½ oz.; left, 4½ oz.; capsules adherent; urine very albuminous.	Within the inner capsule of right cerebral hemisphere was a cavity the size of a pea, no softening. For history see the end of this table.
No. 63, F., 18, housemaid.	A decolourised clot, size of a marble, attached firmly to apex of left ventricle; aorta atheromatous. Fig. 4.	Unrecorded.	Cloudy swelling of cortex; urine albuminous.	Admitted 16 days before death with symptoms of enteric fever.
No. 64, M., 8.	A considerable patch of atheroma on mitral valve; aortic valve reddened.	Numerous embolic infarcts of a dark red colour, size of peas, in lungs.	Cortex congested and swollen; urine albuminous.	Diphtheritic exudation in larynx, etc.; cellulitis of right side of neck; necrotic slough on upper lip; in hospital 2 or 3 weeks.

APPENDIX.—Cases of Atheroma—continued.

Sex, Age, etc.	Vascular System.	Lungs, Pleuræ.	Kidneys, etc.	Other Organs, History, Remarks, etc.
No. 65, M., 11.	Mitral valve œdematous; several streaks of atheroma at commencement of the aorta.	Some congestive œdema of both bases; a tough valvular flap of membrane hanging freely in trachea from mucous membrane of larynx about 2 in. downwards; tracheotomy wound below.	Capsules stripped; intense congestion of pyramids; cortex pale.	Admitted on fourth day of illness, 24 hours before death, with paroxysms of dyspnoea; stridor; diphtheritic exudation on left tonsil; 5 c.c. Ruffer's antitoxin on fourth day of illness; tracheotomy attempted before death.
No. 66, M., 29, furniture remover.	Heart, 11 oz.; some dilatation of cavities; valves fairly healthy; thoracic aorta beyond arch much streaked with atheroma. Fig. 7.	Lungs very œdematous and congested at bases; pleuræ normal.	Weight, 13 oz.; capsules stripped; cortex thick and congested; urine, a trace of albumen.	Skull thick; arachnoid opaque over vault, and cortex congested. For history of this case of hyperpyrexia see at end of this table.
No. 67, M., 3½.	One small patch of atheroma on mitral valve; commencing ulceration of intima at the beginning of thoracic aorta. Fig. 2.	Normal.	Normal.	Died within a week after severe burns over thighs and abdomen; no intestinal ulcers.
No. 68, F., 4½.	Several streaks of atheroma over aorta. Fig. 1. Mitral valve fringed with small vegetations.	Normal.	Normal.	Died 4 days after receiving burns on body, head, and arms; no duodenal ulcers.
No. 69, M., 82, lodging-house keeper.	A soft fungoid growth on intima of aorta, close to the termination of the arch.	Many abscess cavities in upper lobes of both lungs.	Apparently healthy.	Epithelioma of œsophagus; ulcer opened into trachea.
No. 70, M., 69, shoemaker.	"Cat's tongue" lymph on visceral pericardium; several patches of atheroma on descending thoracic aorta.	Recent lymph over both lung surfaces; left lung solid throughout; upper lobe in a state of grey hepatization; lower lobe reddish, breaking down; right lung congested.	Cortex thin; contracted and granular surface; urine contained few chlorides.	Ill 4 days.

HISTORY OF CASE, No. 66.—The patient, whose previous health had always been good, with the exception of influenza 18 months ago, was suddenly attacked with sore throat, and pain, redness, and swelling of the knees, and other joints; for 3 weeks before admission he was confined to bed; on arrival at the hospital he was semi-delirious, with a temperature of 105° F. There was moderate effusion into both knees, a weak, rapid pulse, and some albumen in the urine. The heart's apex was somewhat displaced outwards, and there was a maculated red rash over skin of chest, abdomen, and inner side of arms; in places there were minute pustules. Shortly before death, which took place 2 days after admission, he became wildly delirious, and the temperature rose to 108°·6 F.

THE DURATION OF LIFE IN CASES OF INFECTIVE ENDOCARDITIS.

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Sussex County Hospital.*

Two examples of this disease, in which I have recently had opportunities of verifying the diagnosis at the autopsies, throw some light on the duration of life in these cases. Their histories are briefly as follow. See also Appendix to previous paper, p. 377.

HISTORY OF CASE, No. 61.—The patient, a single woman, had had feeble health for 5 years past, at which date she had an attack of “rheumatic fever,” and was an in-patient of the Sussex County Hospital. She had been subsequently readmitted several times as an in-patient, suffering from heart troubles, pains in joints, epistaxis, vomiting, and so forth. On one occasion she had paresis of some of the muscles of the left fore-arm and hand. When admitted for the last time in September 1894, the heart's apex was found displaced outwards to nipple vertical in fifth interspace; the area of dulness was somewhat increased, and a loud double murmur was heard over the aortic cartilage, and less loudly towards the apex; the bases of lungs were subcrepitant; both spleen and liver were palpable below the margin of ribs; the legs were swollen at times, and the urine, sp. gr. 10·10, contained albumen. The average daily amount passed towards the end of life was 20 oz.; she was then troubled with persistent vomiting. The temperature was variable; on most days it was 3 or 4 degrees above normal for a few hours. She died of exhaustion, 25th October.

HISTORY OF CASE, No. 62.—About 2 years before his death the patient had an attack of “rheumatic gout,” and some weeks subsequently he came under observation as an in-patient with left hemiplegia and heart disease. At the cardiac apex, which was displaced outwards, there was a presystolic murmur. After a 3 months' stay in the hospital, he, to a large extent, recovered the use of his limbs, and was discharged; but he returned again at the end of 5 weeks with paresis of the left arm and hand. This second attack came on suddenly after a “bolster match” with his brother. The heart's apex was 2 in. outside nipple line in fifth space. The presystolic murmur remained; temperature about normal; there was no albumen in the urine. After a month's stay in hospital he was discharged with almost complete recovery of the use of the paralysed muscles. He was finally admitted about a year afterwards, with orthopnoea, œdema of legs, and persistent vomiting. The heart apex beat was in the sixth space, considerably outside the nipple line; its action was hurried and irregular; the pulse, 96, was of low tension; temperature 100° F.; the urine was very albuminous. He died 4 days after the last admission.

One patient, a girl, first came under observation when she was 17 years of age, and was repeatedly seen as an in-patient by me until the time of her death 5 years afterwards. In all she had four severe attacks of illness during that period. Of these the earlier attacks were diagnosed "acute rheumatism," as they usually are when the cardiac symptoms are accompanied with some arthritis. On carefully reviewing the clinical histories of her various attacks I am, however, now confirmed in the opinion that throughout her illness I was watching and treating progressive outbursts of identical pathological processes (using the term in the sense recently adopted by Dr. Semon), for the following reasons. She had heart trouble from the commencement; she had repeatedly, throughout her attacks, pain, redness, and swelling of the knees, shoulders, and hands. At an early date she became subject to epistaxis, and bleeding from the gums. On her penultimate visit to the hospital as an in-patient, 4 months before her death, she had loss of power in the left forearm and hand, due probably to an embolus. And, finally, in her last attack, her symptoms were ascribable to the failure of a long wearied and a much worried heart to carry on the work of the circulation. After death, besides considerable hypertrophy of the left ventricle, there was upon the auricular surface of the aortic cusp of the mitral valve a tough fleshy growth about half an inch long, and an old superficial ulceration of the wall of the left auricle. The aortic cusps were shortened and thickened. There was an old infarct in the right kidney.

The second case, that of a young man, an undertaker by trade, is in many respects similar to the above. The duration of life after the history of an attack of "rheumatic gout" was, however, shorter, certainly not more than two years, if so long. He was an in-patient on three separate occasions. Here again there was progressive heart disease from the first, with the early appearance of unilateral paralysis, which a prolonged stay in the hospital "cured." He soon, however, returned with renewed signs of arterial plugging, to be again discharged cured. In this example of infective endocarditis, unlike the other, the early stages of the disease were not associated with high temperatures and severe arthritic symptoms. There were, however, occasional attacks of pain and swelling of the right ankle, the "rheumatic gout," but there was no history of mucous hæmorrhages recorded in the notes. The final symptoms, the breakdown in the cardiac muscle, the persistent vomiting, the anasarca, the albuminous urine, and the congested lungs were closely alike in both instances. The autopsy showed from the size and weight (23 oz.) of the heart of the youth, that the disease of the valves was a chronic one, a conclusion confirmed by the clinical history. It also showed the heart at the time of death to have been pathologically in a quiescent state; that is to say, the firmly coherent mitral flaps with their contracted orifice, fringed by short fleshy growths, were the passive results of an

acute process, which had then ceased. Infective endocarditis consists, I take it, essentially of a series of sudden outbursts of acute disease, followed by prolonged intervals of healthy calm, during which a youthful patient can apparently repair more or less fully any structural flaws, induced by the acute attacks.¹ If this view of the disease is admitted to be the true one, and I cannot find any other interpretation so applicable to the various clinical and pathological facts of cases under my care, the resemblance between infective endocarditis and acute rheumatism, in these as in many other respects, is close and striking. Infective endocarditis is, however, admittedly a disease associated with the presence of microbes within the vascular system, whilst the relationship between these bodies and acute rheumatism is at all events doubtful. As fatal cases of acute rheumatism without heart mischief must be exceedingly rare, and will include inflammations of the lungs and pleural cavities when pneumococci usually abound, the etiological uncertainty of this disease is readily explained.

The student upon his first visit to a bacteriological laboratory cannot fail to note how difficult it is to obtain a pure culture of a bacillary growth. What untiring vigilance has to be exercised, what elaborate precautions have to be taken by the manipulator to prevent the introduction into the nutrient jelly of other germs than those he seeks to cultivate. Much of the difficulty, I admit, has been overcome by routine procedure, yet this only emphasises the fact that nature in her profusion sows the face of the jelly with air-borne germs of many species, unless she is prevented by man. The valvular growths found within the heart in infective endocarditis are admittedly the home of many different species of bacteria in different subjects.² Not only is this the case, but several species of cocci may occur in the same subject. And this bacterial commixture of species is not confined to the endocardial growths, it is common to other diseases, to the teeming membranes of diphtheria, and the rice-water stools of cholera;³ as we might expect from the experience of the laboratory. There is, however, this important distinction between these examples of mixed infection. In the two last the culture beds of the microbes within the body may be, and probably often are, directly contaminated through the air; whilst the first, by the position of the heart and the great

¹ Drs. Pye-Smith and Frederick Taylor, amongst other writers, have recorded cases of so-called recovery from infective endocarditis. The most curious part about these recorded cases is their rarity, and for this result the nomenclature of the early stages of the disease is largely responsible.

² Tubercle bacilli, the *Diplococcus pneumoniae*, the anthrax bacillus (Oliver), Typhus bacilli (Dreyfus-Brisac), besides the streptococci and staphylococci of pus, have been found by different observers within the heart or aorta in this disease. Fränkel and Sängner found five different pathogenic microbes in thirteen cases of endocarditis (ulcerosa and verrucosa). Kanthack says there are at least twelve different organisms met with in ulcerative endocarditis!

³ Diphtheria (Klebs-Loeffler) bacilli are frequently associated with pus cocci, *Spirillum cholerae asiaticæ* with Finkler-Prior spirillum (Weichselbaum).

vessels, removes its culture beds as far as possible from all sources of direct contamination; yet we know that even here within the heart, whither germs can only be conveyed by the hæmolymph system, mixed infection so frequently occurs as to be the rule rather than the exception. It would seem probable that as soon as a swarm of pathogenic microbes have succeeded in forcing a passage through the body's chief lines of defence against such attacks, namely, the skin and the mucous membrane, others of the same or of different species can readily follow.

If the distinguishing peculiarities of each mycotic disease is due to some specific differences in the pathogenic microbe,—its etiological factor,—as some bacteriologists assume, the wonder is, when we consider the ease with which a mixed infection can take place that these specific disorders have any constant characters of their own, not that the characters occasionally vary. These remarks apply especially to cases of infective endocarditis, where there is apparently no specific microbe, and where, nevertheless, we find "pathological identity associated with great bacterial diversity." In a disease of this character it is not surprising to meet with symptoms varying greatly in different patients. It must also be admitted, I believe, that infective endocarditis develops different clinical features at different phases of its evolution in the same individual. If this be so, the onset of infective endocarditis will frequently date from the first attack of "acute rheumatism," "chorea," "quinsy,"¹ or other affection, at which the earliest "heart symptoms" were detected.

The two cases above quoted go far to show that this disease has periods of pathological calm, in which the recuperative powers of a patient may to some extent repair the damage produced during the active bacterial stage. As time goes on, and the patient's strength becomes more and more undermined, the intermissions usually become shorter and less complete, whilst the active stages are prolonged in duration, although seemingly less acute pathologically. If we date the commencement of infective endocarditis from the final act of this life's tragedy, as is usually done, we obtain an inaccurate, and, I consider, an altogether mistaken conception of the clinical features of this insidious malady. The assumption that the final breakdown of the circulatory apparatus in such cases is due to the sudden invasion of bacteria among the diseased tissues of a previously

¹I have recently had a case of chorea with endocarditis at the hospital. The patient, a girl aged about 10 years, had hypertrophied tonsils. During her stay under my observation she had two attacks of tonsillitis, with high temperatures, and on each occasion an enlargement and some tenderness of the cervical gland at the right angle of the jaw. Dr. Woodhead has emphasised the important fact that the two rings of lymphoid tissue, surrounding respectively the entrances to the gullet and the windpipe, may furnish channels of infection in tuberculosis. It seems possible from the above case that in other diseases also the tonsils may serve as channels for the conveyance of the infection to the central organs.

aseptic heart, is one that cannot be substantiated either clinically, pathologically, or bacteriologically. Cases of infective endocarditis generally recover from the first attack, and, under favourable conditions, may live on for many months and occasionally for years.

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THE EXPERIMENTAL PRODUCTION OF ANÆMIA IN DOGS.

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IN the course of some investigations into the etiology of different forms of anæmia I made two experiments on dogs, which it may be of interest to give in detail, as the condition produced presented some features resembling chlorotic anæmia, and some differing from it materially. My endeavour was to induce in the dogs a morbid state similar to chlorosis, in one of them by bleeding, and in the other by feeding on a diet containing insufficient iron, two factors which, I am convinced, play a very important part in the causation of chlorosis in the human subject.

EXPERIMENT 1.—A dog, weighing $11\frac{1}{2}$ kilogs., was repeatedly bled from a vein in the leg, and observations were regularly made on the condition of the blood by means of Gowers' hæmoglobinometer and hæmacytometer. Such a dog should have, approximately, about 1000 c.c. blood, and in 3 weeks 687 c.c. were withdrawn, and then it was left to recover without any treatment. During the whole time it was fed daily on 200 grms. oatmeal, 1 pint of milk, and salt—a diet which is sufficient to maintain a dog in good health, and which contained about 8 mgrms. of iron. It occasionally got, in addition, scraps of bone and meat, so that the daily ingestion of iron would probably be from 8 to 10 mgrms.

On each day the corpuscles and hæmoglobin were estimated *before* the animal was bled, and 108 and 8,000,000 were taken as the normal quantities of hæmoglobin and red corpuscles respectively, in estimating the richness in hæmoglobin of the individual corpuscles.

On February 20 the dog was killed with chloroform. All the organs were healthy, and there was no fatty degeneration. The liver gave no staining with ammonium sulphide, and on estimating the iron in it only 0.015 grms. Fe. per 100 parts dried was found. The spleen gave a greyish-green colour, with ammonium sulphide; the bone marrow was very red to the naked eye, but no microscopic examination was made.

The details of the blood-condition are given in the following table :—

Date.	Per cent. Hb.	Red Corpuscles.	Hb. Value of each Corpuscle.	Bled to	Remarks.
Oct. 24	108	8,000,000	1.0	30 c.c.	Corpuscles well formed.
" 25	108	8,000,000	1.0	85 "	
" 29	94	6,980,000	1.0	92 "	
" 31	84	5,740,000	1.06	40 "	Corpuscles well formed, a good many microcytes; sp. gr. of serum, 1.012.
Nov. 1	78	6,300,000	0.92	130 "	
" 3	68	6,280,000	0.80	70 "	Sp. gr. of blood, 1.041; of serum, 1.022.
" 7	60	5,400,000	0.82	120 "	
" 12	50	5,640,000	0.65	120 "	Sp. gr. of blood, 1.038; of serum, 1.024.
" 16	48	5,280,000	0.66	687 c.c.	
" 21	48	5,500,000	0.62		Slight poikilocytosis.
" 28	52	6,020,000	0.64		Corpuscles very pale, many some- what misshapen, and a few nu- cleated.
Dec. 6	52	6,300,000	0.61		
" 13	52	7,800,000	0.50		
" 20	54	8,700,000	0.46		
" 28	62	9,200,000	0.50		
Jan. 3	72	10,000,000	0.52		
" 17	90	11,200,000	0.60		
" 30	98	10,400,000	0.69		
Feb. 20	104	10,320,000	0.74		

On looking over the experiment, it will be observed that the red corpuscles and hæmoglobin diminished at first in equal proportions, but in a very short time the deficiency in hæmoglobin became much the more marked, and after the last bleeding, when both were at their lowest ebb, the hæmoglobin had sunk to less than half its original amount, while the corpuscles had fallen only about one-third. In one month the corpuscles had returned to their original number, the hæmoglobin having by this time gained only 4 per cent., and three months elapsed before the latter had returned even approximately to its original amount. There was some poikilocytosis with a good many microcytes, and the blood serum was little altered in specific gravity, although the specific gravity of the blood fell considerably, thus indicating that the deficiency was in the corpuscles. The hæmoglobin value of the individual corpuscle also became very low.

All this is similar to the blood condition found in chlorosis, and if the experiment had been stopped sooner than it was, the impression would have been produced that a condition exactly similar to chlorosis had been induced in the dog. But the animal went on producing more red corpuscles, until at one point the number reached over 11 millions per cub. mm., a state of affairs never seen in chlorosis, where the corpuscles are frequently normal in number, but

more usually are deficient. The animal never developed heart murmurs or breathlessness, and throughout appeared to enjoy the best of health.

It has now been well established that, after bleeding, the red bone marrow of dogs hypertrophies and rapidly produces new corpuscles, and this accounts no doubt for the increase of corpuscles over the normal, and for the fact that they do not diminish rapidly in number as they have time to regenerate in the intervals between the bleedings. The hæmoglobin, however, is in a different position, as the reserve supply of iron in the liver and other organs is soon used up in making it, and the iron of the food (only about 8 mgrms. per day) is not sufficient for the greatly increased manufacture of red blood corpuscles. Hence the hæmoglobin returns very slowly to the normal, and the hæmoglobin value of each red disc remains extremely low, while it takes a very long time to re-establish the balance between corpuscles and hæmoglobin. After death the dog's liver contained much less than the usual amount of iron, showing the drain there had been upon it.

EXPERIMENT 2.—A dog, weighing $12\frac{1}{2}$ kilogs., was fed on a diet of starch, lard, milk coagulum, and salts, from all of which the iron had been removed as completely as possible. It got as much of this as it cared to eat daily, which furnished it with 2 to 3 mgrms. of iron per diem. Previous to commencing this diet, it was fed for a fortnight on skim milk—a diet poor in iron—with the object of exhausting the reserve iron in its liver and other organs. In calculating the hæmoglobin value of each corpuscle, 90 and 6,000,000 were taken as the normal value of the hæmoglobin and red corpuscles respectively.

Date.	Per cent. Hb.	Red Corpuscles.	Hb. value of each Corpuscle.	Remarks.
Jan. 10	90	6,000,000	1·0	Put on diet containing 2-3 mgrms. iron per day.
„ 21	80	6,000,000	0·88	
„ 28	74	6,800,000	0·72	
Feb. 12	66	6,250,000	0·70	Put on diet containing about 10 mgrms. iron per day—oatmeal milk and scraps.
„ 25	64	6,800,000	0·62	
March 4	65	6,500,000	0·66	
„ 11	68	6,500,000	0·69	
„ 22	70	6,400,000	0·72	
April 9	76	8,000,000	0·62	
May 3	76	8,000,000	0·62	

Dog killed. Its organs were found healthy. During the experiment the dog kept its weight and remained in good health.

During the 46 days the dog was fed on the diet poor in iron, the hæmoglobin had diminished about one-third, while the red corpuscles

had slightly increased in number, and afterwards continued to do so until the increase was very marked, namely, one-third of their original number. Except for this increase in the number of red discs the condition of the blood was similar to what is found in chlorosis.

It is evident that during the first part of the experiment the excretion of iron must have been greater than the ingestion, and that there was not enough iron in the dietary to furnish sufficient hæmoglobin for any new corpuscles formed. During the second stage more than enough iron was furnished, as the hæmoglobin increased in amount, but only very slowly, as it required 67 days for the hæmoglobin to rise from 64 to 76 per cent. on Gowers' scale. It is the invariable experience that in recovery from chlorosis the hæmoglobin increases much more slowly than the corpuscles, whether medicinal preparations of iron be given or not. In the human subject, in chlorosis, recovery takes place with extreme slowness, or more usually not at all,¹ when purely dietetic treatment is used owing to the very small amount of iron in ordinary dietaries; hence it is necessary to give iron in addition.

While clinical observation has led me to the conclusion that blood loss (usually during the menstrual periods) and insufficient iron in the dietary are the immediate causes of chlorosis, yet by these means the condition cannot be exactly simulated in dogs, as these animals immediately produce an excess of corpuscles as if to make up for the deficiency in hæmoglobin, and this we never find in cases of chlorosis. There must therefore be some cause or causes superadded in the human subject. As chlorosis occurs almost invariably during the years of growth, when an extra strain is thrown on all the functions of the body, which is often very inadequately met in many directions, it seems to me that this may limit the blood-making power as well as impair the digestion and general nutrition, so that these causes combine with those above mentioned to produce the pathological conditions of chlorosis.

¹ Cf. Stockman, "The Treatment of Chlorosis by Iron and some other Drugs," *Brit. Med. Journ.*, London, 1893, vol. i.

THE EXCRETION OF OXALIC ACID IN URINE, AND ITS BEARING ON THE PATHOLOGICAL CONDITION KNOWN AS OXALURIA.

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THE following observations were made and collected during the carrying on of an investigation for the purpose of explaining, as far as possible, the pathology of the condition known as oxaluria. "Oxaluria" is a condition about which opinions differ widely. Some physicians are very ready to diagnose it, and consider that all dyspeptics whose urines deposit crystals of oxalate of lime are really suffering from oxaluria; while others consider oxaluria to be a strictly limited pathological condition. I was impressed by this difference of opinion by being told by a physician, that more than half of the cases of dyspepsia which passed through his hands were suffering from oxaluria; while on referring to the records of the Royal Infirmary, Edinburgh, I find that out of 700 cases of dyspepsia admitted, only three were diagnosed as oxaluria. Such a difference of opinion can only be explained by the want of accurate knowledge as to the significance of the presence of crystals of oxalate of lime in the urine.

The physiological aspects of this subject are equally uncertain. Previous observations are of a most contradictory kind, and although several theories have been advanced, no one of them is generally accepted as explaining why oxalic acid is excreted in the urine, or why the amount of the excretion should vary.

Under these circumstances I trust that the new facts I have been able to gather may be of value.

I shall first consider the history of oxaluria, then its physiology, and, lastly, the application of the physiological conclusions to the pathological condition known as oxaluria.

HISTORICAL.

Oxalate of lime was first recognised in the urine by Wallaston (⁹⁹) in 1797; he found it to be a constituent of some calculi, and described

it as always being associated with phosphates and uric acid. Two years later Fourcroy ⁽²⁶⁾ discovered oxalate of lime in the sediments which collect on the inside of urinals, showing that it occurred independently of calculus formations. These observations lead to others being made. Of these, the earlier ones, such as those of Brandes ⁽¹⁴⁾, Vauquelin ⁽³⁹⁾, Gaultier ⁽³⁵⁾, Martin et Prevost ⁽⁵⁷⁾, Lassaigne ⁽⁵⁰⁾, all referred to the formation or analysis of urinary calculi. The first mention of an "oxalic" diathesis was made by Prout ⁽⁶⁴⁾ in 1827. He describes oxalates as occurring as an "amorphous sediment, very rarely crystalline, and sometimes mixed with urates." Some time later, Vigla ⁽⁹¹⁾ studied the octohedral crystals, which had previously been found in urine, and which had been credited with being crystals of chloride of sodium, and argued from the fact that in urine there is not sufficient chloride to allow of its being crystallised, that these octohedra are not chloride, but must be something else. What they are he did not ascertain. It was not till 1839 that these crystals were shown to consist of oxalate of lime, and were accurately described. This was done by Donné ⁽²²⁾, who compared them to crystals of chloride of sodium, but pointed out that they were much less soluble, and consisted of two pyramids joined together by their bases; and that they were insoluble in acetic acid, but soluble in nitric acid without effervescence. He also observed that to produce them in urine all that is necessary is to eat a considerable quantity of sorrel. The crystals were further studied by Bret ⁽¹⁵⁾ and Willis ⁽⁹⁷⁾. Golding Bird ⁽³⁷⁾ was the first to point out their frequency as a urinary deposit, this he did after a long series of observations. His method was to allow the urine to stand for 24 hours in a urine glass, decant off the upper part and put some of the lower part in a watch glass, heat it, let it stand, then remove most of the fluid with a pipette and replace with distilled water, when bright particles—the crystals—became evident. These were examined under a microscope with a half-inch power. This method is obviously fallacious, as the boiling would render most urines alkaline, and this would cause a deposition of earthy phosphates, which would be indistinguishable from oxalates under a low power of the microscope.

Golding Bird's conclusions were, that it occurred in many conditions, but was especially associated with instability of the nervous system. He describes the symptoms accompanying the excretion of oxalates, mentioning anæmia, hypochondriasis, want of energy, and pains in the loins. He mentions as causes of their excretion, persistent derangements of health, previous acute disease, dyspepsia, syphilis, mercurialism, and over-lactation.

A few years after this, Begbie ⁽⁸⁾ published in the *Monthly Journal of Medical Science* his well-known paper on oxaluria. He describes the symptoms of oxaluria, and says it is caused by "a poison, produced during digestion and assimilation, carried into the blood by the ordinary channels, but limited in its pernicious consequences by the busy agency

of the urinary organs in separating it from the circulation and discharging it from the system. By this elimination we are enabled to detect the offending matter in the urine in the form of oxalate of lime."

Dr. Begbie also points out that the presence of oxalate in urine is alone insufficient to warrant the diagnosis of the "oxalate diathesis." "I am aware," he writes, "that oxalate of lime is found in the urine in circumstances very different from those described. That it is found in all ages, both sexes, and very different conditions of life. That it is to be found as an immediate cause of a fit of gravel, where no mal-assimilation has been prominently manifested, and that the records of the public institutions for the management of the insane show that it is very common in the urine of those suffering from melancholia.

I limit my remarks at present to a class of cases of a well-marked character, in which its presence is a prominent feature, and from which important indications of diagnosis and treatment may be derived."

Begbie's paper was followed by many others,¹ some giving cases, others referring to special points, while others refused to recognise such a disease and criticised severely all the work done.

Among these latter is a long and careful paper by Gallois⁽⁸³⁾, in which he considers the whole subject, criticises a large number of the published cases, and comes to the following conclusion:—

"1. Oxaluria is frequently met with in the urine in health, at all ages, and in all conditions of life.

"2. Its appearance is influenced by certain food-stuffs and by certain drugs.

"3. It is often found in the urine of patients suffering from very different diseases. Oxaluria, then, is not a pathological entity, but a symptom common to very different diseases. It is frequently associated with spermatorrhœa, diseases of the nervous system, and especially with dyspepsia."

Dr. Begbie's work, however, has never been fully reinvestigated, and oxaluria has, up to the present, been considered a special disease. Recent work has been directed mainly to the explanation of the various phenomena of this disease and its etiology. I shall refer to these *seriatim* in considering the subject.

PHYSIOLOGY OF THE EXCRETION OF OXALIC ACID.

A. *Frequency of Occurrence of Oxalic Acid in Urine.*

The first question which I shall discuss is, *the frequency of the occurrence of oxalic acid in the urine.*

Previous to the use of the microscope in practical medicine very little was known about this, but with its help observers were able to recognise oxalic acid in a large proportion of urines. This proportion has

¹ (3), (4), (5), (6), (7), (11), (24), (26), (51), (55), (60), (82), (87), (94), (96).

been very variously stated, but the figures I shall reserve till I discuss the cause of its precipitation. The microscope, however, has not been able to answer the question entirely. With the growth of the use of chemistry, analytic methods were used, and, as the result of a series of most tedious analyses, it has been shown by Fürbringer (³²) that oxalic acid is a normal and almost constant constituent in the daily urine of a healthy man.

It might be argued against this conclusion that the number of urines examined by so tedious a method is necessarily too limited for the deduction of such a general statement, but it is fully corroborated by a method of qualitative examination suggested by Dr. Reoch (³⁰), which I have used in examining a large number of urines.

The method depends on the fact that oxalate of lime is insoluble in alcoholic solutions, so that by the addition of alcohol to urine we get any oxalate that may be present precipitated.

The procedure which I have adopted is to partly fill a sediment glass with urine, and quietly pour alcohol on the surface of it, in sufficient quantity to nearly fill the glass. The object of doing this by the "contact method" is to allow the alcohol to mix slowly with the urine, so that the precipitated oxalate may be as perfectly crystallised as possible, and so be more ready of recognition under the microscope. The glass with its contents is allowed to stand for 24 or 48 hours, and then the sediment is lifted by a pipette for microscopic examination, or the sediment along with the lower part of the fluid is collected in a pipette, put into the tubes of a cream-testing centrifugal machine, centrifugalised, and the sediment so obtained examined. This second way is necessarily more thorough, and is the one I have always used.

The examination by this method of a large number of urines, both of healthy adults and of hospital patients, enables me to corroborate the results got from quantitative analysis, namely, *that in the urine of men eating an ordinary mixed diet, oxalic acid is a normal and constant constituent*. I have examined upwards of a hundred specimens with the method, and have found crystalline oxalate of lime in all of them, except in those of patients getting an absolute milk diet. I shall fully consider this exception later.

B. *On the Precipitation of Oxalate of Lime.*

Oxalic acid being a constant constituent in urine, the question which naturally suggests itself is, *Why is it not always precipitated in the form of oxalate of calcium?* In order to answer this question, I shall consider the influence of various factors on the solution of oxalic acid in urine, the proportion of urines in which precipitation occurs, and what the essential difference is between urines depositing oxalate of lime, and those not doing so.

a. Factors influencing the Solution of Oxalic Acid in Urine.

1. *The amount of calcium.*—That this is a factor which might influence the solution or precipitation of oxalic acid in urine is evident from the very insoluble nature of the calcium salt of oxalic acid, and it might be presumed that it is owing to excess or deficiency of calcium salts in urine that the oxalic acid is allowed to remain in solution or is precipitated. I find that this is not the explanation, as there is invariably more than a sufficiency of calcium in urine to precipitate the oxalic acid present. This can be demonstrated by simply adding some solution of oxalic acid to urine, and, in all cases, this is followed by a precipitation of oxalate of calcium. I have made this observation frequently with unvarying result, I am therefore forced to the conclusion that it is no deficiency of calcium that permits the solution of oxalic acid in urine. That this is so is also made apparent by the fact that the daily excretion of oxalic acid amounts, on an average, to only 0.0172 grm., while the amount of lime excreted daily is about 0.339,¹ a quantity about twenty times as great as the quantity of oxalic acid.

There, then, being both calcium and oxalic acid in urine, where no precipitation of oxalate of calcium occurs, it becomes necessary to try and find out what substance in urine is capable of preventing this precipitation.

2. *Acid sodium phosphate.*—Moddermann (⁵⁸) argued from the fact that oxalate was precipitated slowly from urine, or, in other words, that it was precipitated when the acid phosphate of soda was reduced in quantity by being converted into the neutral or alkaline phosphate, that the acid phosphate of soda is the solvent for the oxalate of calcium. This explanation is generally accepted, but, as I have found no direct observations on this point, I have investigated it in the following manner. Two beakers were taken, A and B; in A there was put some distilled water, in B a similar quantity of a solution of pure acid phosphate of soda; to each was added a similar quantity of solution of chloride of lime, and then a similar quantity, very carefully measured, of oxalic acid solution. The beakers were allowed to stand for 24 hours, and then the precipitates which had formed were collected on ash-free filter papers, washed and reduced by the flame of a blow-pipe to quicklime; from the weight of the lime so recovered was calculated the amount of oxalic acid in the precipitate. This I did six times, and each time the weight of oxalic acid precipitated in Beaker B was less than that in Beaker A; from this I concluded that the acid phosphate of soda had kept some of the oxalic acid in solution, notwithstanding the fact that there was present an excess of a lime salt. The weights obtained in these six observations are shown in the following table:—

¹ Bunge, "Physiological Chemistry."

Table showing Solvent Action of Acid Sodium Phosphate.

	Beaker A.	Beaker B.	Weight of Oxalic Acid retained in Solution in Beaker B.
	Water.	Acid Phosphate of Soda Solution.	
1	·135	·133	·002
2	·137	·135	·002
3	·0304	·0284	·002
4	·0096	·0068	·0028
5	·0246	·0230	·0016
6	·0243	·0227	·0016

The acid phosphate of soda solution is not a powerful solvent for oxalate of lime, but is sufficiently so to explain the solution of the small quantity of oxalate which occurs in urine. In the above observations, 5 and 6, 100 c.c. of 1 per cent. solution was used, and it will be seen that the amount of oxalic acid held in solution was only ·0016 grm. in each case, certainly a very small amount; but 2 litres of such solution would contain ·032 grm. of oxalic acid, which is more than the average quantity in a similar quantity of urine.

These observations, while showing that acid phosphate of soda is a slight solvent for oxalates, by no means prove that it is the only solvent which is present in urine—a point of great importance when considering the quantitative estimation.

3. *Urea and chlorides.*—I have investigated in a similar manner the possible action of two chief constituents of urine—urea and chloride of sodium—and my results in both cases were negative. In both cases the weight of lime in Beaker B was as large as that of Beaker A; in Beaker B there being present the urea or chloride of sodium in the same manner as was the acid phosphate of sodium in the above observations.

The following tables show the results of these observations with sodium chloride and urea :—

Table showing effect of Chloride of Sodium as a Solvent for Oxalates.

	Beaker A.	Beaker B.
	Water.	Chloride of Sodium Solution.
1	·0030	·0030
2	·0220	·0227

Table showing Effect of Urea as a Solvent for Oxalates.

	Beaker A.	Beaker B.
	Water.	Urea Solution.
1	·0057	·0060
2	·0220	·0227

β. Proportion of Urines Precipitating Oxalates.

That the solvent action of urine on oxalates is not a powerful one is evident from the fact that in a very considerable percentage of urines, oxalate of lime crystals are found. This percentage is variously stated by different observers, and is probably very far from being a fixed one, owing to the different mode of living and different dietary of the classes of cases examined by the various observers. The largest series of observations recorded are those of Walshe (⁹⁴), Gallois (³³), Baron, and Smoler (⁸⁴), and these give the percentage of urines which deposit oxalate of lime as being between 28 and 50. The exact number stated by each is to be seen in the table which follows. My own observations show that oxalate is precipitated in rather more than *one urine out of three*, for I find in my notebook that out of 126 urines, examined microscopically, oxalate of lime was recognised in 44, which is equivalent to 35 per cent. or rather more than one-third of the total examined.

Table showing Percentage of Urines depositing Oxalate, as stated by the various Authors.

Walshe	28 per cent.
Gallois	36 "
Baron	41 "
Smoler	57 "
Dunlop	35 "

The large percentage of urines which deposit crystals of oxalate of lime must of course be taken into consideration when estimating their diagnostic value.

γ. Is Deposition due to Excess of Oxalic Acid.

The essential difference between urines depositing and those not depositing oxalate of lime is a point which has not previously been demonstrated. It naturally must be due to one of two factors, either an imperfect solvent action of urine, or an excess of oxalate. Fürbringer, (³²) from a long series of analyses, comes to the conclusion that a precipitation does not indicate any increase in the amount of oxalic acid present, which points at imperfect solution as the cause of

its precipitation in so many urines, and this opinion is at present the accepted one.

My observations do not corroborate this conclusion, as I find that where oxalate is precipitated there is almost invariably a *larger percentage* of oxalic acid present than where this is not the case.

In the following tabular statement of the result of my observations on this point, it will be observed that nearly all the urines depositing oxalate contain more than $\cdot 0015$ per cent. of oxalic acid, while those not depositing have nearly all of them less than $\cdot 0017$. There are certainly exceptions, notably Numbers 3 and 4 in the series. These may be due to error of observation, the crystals being overlooked in microscopic examination, or they may be due to the presence of some other solvent which I have not been able to recognise :—

Table showing Percentage of Oxalic Acid in Urine.

	Those Depositing Oxalate.	Those not Depositing Oxalate.		Those Depositing Oxalate.	Those not Depositing Oxalate.
1	$\cdot 0093$...	17	...	$\cdot 0014$
2	$\cdot 0032$...	18	$\cdot 0013$...
3	...	$\cdot 0022$	19	...	$\cdot 0013$
4	...	$\cdot 0021$	20	...	$\cdot 0012$
5	$\cdot 0020$...	21	...	$\cdot 0010$
6	$\cdot 0020$...	22	...	$\cdot 0010$
7	$\cdot 0019$...	23	...	$\cdot 0010$
8	$\cdot 0019$...	24	...	$\cdot 0009$
9	$\cdot 0018$...	25	...	$\cdot 0009$
10	$\cdot 0017$...	26	...	$\cdot 0006$
11	$\cdot 0017$...	27	...	$\cdot 0005$
12	...	$\cdot 0017$	28	...	$\cdot 0003$
13	$\cdot 0016$...	29	...	$\cdot 0003$
14	$\cdot 0015$...	30	...	$\cdot 0002$
15	...	$\cdot 0015$	31	...	$\cdot 0002$
16	...	$\cdot 0015$			

Table showing Averages of above.

	Total in Series.	Depositing Oxalate.	Not Depositing Oxalate.
Number . .	31	12	19
Average percentage of oxalic acid contained .	$\cdot 0013$	$\cdot 0025$	$\cdot 0010$

The estimations from which these calculations were taken were made by a new method which I shall presently describe.

From these analyses I infer that a *precipitation of oxalate of lime* usually indicates a *high percentage of oxalic acid in the urine*, but this does not necessarily indicate an increase in the amount of oxalic

acid present in the daily urine, as this depends not only on the percentage of oxalic acid present, but also on the quantity of urine passed. However, with a urine depositing crystals of oxalate and of a daily quantity up to the average amount, one may assume a comparatively large excretion of oxalic acid.

C. *Form of Oxalate of Lime Crystals.*

Oxalate of lime is generally described as occurring in urine in two distinct forms, each of these having some variation.

Octohedra.—The ordinary and, as I believe, the only form, occurs as octohedral crystals consisting of two flattened pyramids joined together by their basis, which, when seen under the microscope, tend to lie on one of the facets, and thus get the appearance which is compared to an envelope. These crystals are highly refractile, have sharp, well-defined angles, and are of varying size, their long—that is the oblique—diameter being from 3 up to about 20 mm. These crystals are also seen lying on their edge, and then appear to be elongated, and lozenge-shaped, their long diameter being about three times the length of their short diameter. That these octohedral crystals do consist of oxalate of lime there can be no doubt, as they can readily be produced artificially by adding solution of oxalic acid to a solution of lime salt. Further, the crystals in urine have the same solubilities as those artificially prepared; they are not dissolved by heat, are insoluble in acetic acid, but are soluble in nitric and hydrochloric acids without effervescence.

Dumb bells.—The other form in which oxalate of lime is described as occurring in the urine is as dumb-bell crystals—oval crystals with a constriction round their centre. Hugh Balfour⁽⁵⁾ first described these crystals as being composed of oxalate of lime, and deduced this from the fact that he frequently found them deposited in the urines where the octohedral crystals were deposited; his conclusion does not seem to have ever been doubted. Large and beautifully-formed dumb-bell crystals are seen in horses' urine, and it is there that I have examined them. I find that they differ essentially from oxalate crystals in being soluble in acetic acid, and, further, that this solution is accompanied by effervescence, showing that these *dumb-bell crystals are not oxalate at all*, but are composed of carbonate. I have verified this observation with dumb-bell crystals in human urine. Vesque⁽⁶⁰⁾, in a careful paper, studies the crystallisation of oxalates, and he remarks on the great difficulty in the production of dumb-bells; he states that they have only been produced by adding potassium oxalate and chloride of calcium to albuminous solution—he does not mention any chemical analysis of them; and I consider it quite probable that here too they may consist of carbonate of lime, the carbonic acid being formed during the decomposition of the albumen. It is also to be noted that now that urine is much more

carefully collected and preserved than formerly, and consequently not in the same advanced state of decomposition when examined, dumb-bell crystals are much more seldom met with; and from this it may be inferred that they arise from decomposition; in decomposition there is no evidence of formation of oxalic acid, while there is undoubtedly formation of carbonic acid, urea being converted into ammonium carbonate.

My conclusion regarding the form of crystal in which oxalate of lime is met with in urine is, that it occurs as *octohedral crystals only, the octohedra appearing as square envelope shape, or long lozenge shape, according to the position in which they are lying.*

D. *The Amount of Oxalic Acid present in Urine.*

Quantitative estimation of oxalic acid in urine is, on account of the small quantity present, and of the complex composition of urine, a difficult and tedious process. So tedious and lengthy are the processes of estimation which have been described that they can hardly be used for the purposes of clinical medicine, and the series of observations from which deductions have been made are all necessarily limited in number.

a. *Methods.* Four methods have been described, and to these I now add a fifth.

1. Lehmann (⁶²), in 1853, was the first to attempt the estimation. His method was to evaporate urine, extract with alcohol, wash the extract with ether, and then estimate the oxalic acid contained in the ethereal extract. This method has been shown to be fallacious, as the urine after being so extracted contained oxalic acid.

2. Schultzen (⁷⁸) was the next to describe a method of estimation; he rendered the urine alkaline by adding ammonia, then added excess of calcium chloride to precipitate the phosphoric acid, and then evaporated it on a water-bath; the resulting residue he washed with strong spirit of wine, with ether, and then with absolute alcohol; this left a yellow powder, consisting of sulphates, urates, phosphates, and oxalates; this was washed with water to remove the sulphates, and with dilute acetic acid to remove phosphates; the oxalates were then separated from the urates by dissolving them in hydrochloric acid. From this solution the oxalates were precipitated by adding ammonia in slight excess, and then rendering the solution acid with acetic acid; the precipitate was then collected on a filter paper, washed and weighed. The results obtained by this method were too high, as there was always some phosphate of lime left and weighed along with the oxalate. Schultzen stated that the average excretion of oxalic acid is .07 grm. per diem; in one case he found as much as .5 grm. present in 24 hours; the urine being that of a patient suffering from jaundice. I mention this figure, as it is frequently quoted as the maximum which has ever been found, but it must be noted that this maximum was ascertained by means of a method which is now abandoned, and which is probably incorrect.

Löbisch (⁶⁴) has recently suggested a modification of this method,

and describes it in the latest edition of his work on *Analysis of Urine*; instead of precipitating the oxalic acid a second time, he estimates the amount in solution by titrating with a standardised solution of potassium permanganate. I tried this modification of Schultzen's method in a few cases, but as I did not obtain satisfactory results I abandoned it. I have seen no criticism of it, and have not seen notes of any observations in which it has been used.

3. Neubauer's ⁽⁵⁹⁾ method is much more accurate. Up till now it has been considered the best and most trustworthy method of analysis. A large quantity of urine is taken, about half a litre is a convenient amount, and to it is added excess of chloride of calcium, then sufficient ammonia to render it alkaline; with this a copious precipitation of phosphates takes place, acetic acid is then added till the solution is rendered slightly acid. This re-dissolves the phosphates, while any oxalate present, if previously precipitated, is not re-dissolved, and if not previously precipitated will crystallise out; the urine is allowed to stand in this condition for 24 hours; it is then filtered, and the precipitate washed with large quantities of water and dilute acetic acid; the oxalate is then separated from the remaining sediment—mucin, epithelium, uric acid—by dissolving it in hydrochloric acid. From this solution it is precipitated by neutralising with ammonia. It is then collected on an ash-free filter paper, washed and incinerated in the flame of a blowpipe, to reduce the oxalate of lime to quicklime; this is weighed, and from it can be calculated the weight of oxalic acid recovered. The method, with use, has been slightly modified in detail.

The accuracy of this method has been tested by Czapek ⁽²¹⁾, and his conclusion is, that the results obtained are only 5 per cent. too small. His method of testing its accuracy was to add a known quantity of oxalic acid—this quantity being estimated by titrating a solution of pure oxalic acid with normal soda solution—to water, and also to samples of urine, in which the amount of oxalic acid contained had been previously estimated, and then to estimate the amount of oxalic acid recoverable by the method. The results of his observations I have represented in the following table:—

Table showing Results of Czapek's Observations to test the Accuracy of Neubauer's Method.

	Medium used and Quantity.	Oxalic Acid Added.	Oxalic Acid Recovered.	Deficiency.
1	Urine, 500 c.c.	·020 grm.	·0197	·0003
2	Urine, 500 "	·020 "	·0191	·0009
3	Water, 400 "	·020 "	·0184	·0016
4	Water, 500 "	·020 "	·0198	·0002
5	Water, 200 "	·020 "	·0189	·0011
Total,	·100 grm.	·0930	·0041

This method of testing the accuracy is incomplete, for were there a residue of oxalic acid left in solution in the urine, to which no additional oxalic acid was added, then the same residue would be left in the other observation, and it would not be made evident, consequently this test does not show that the original oxalic acid of urine is all or nearly all recovered.

I have attempted to verify the accuracy of this method by applying it to a solution of oxalic acid of known strength in acid sodium phosphate solution, but have failed to do so, as calcium phosphate was always found precipitated as large crystals, extremely difficult to dissolve or separate from the calcium oxalate, and in all my attempts the weighings which I obtained exceeded the weight of oxalic acid added. We have then no proof of the accuracy of this method.

I have used Neubauer's method in a long series of observations, and have been struck by unaccountably high results cropping up without apparent cause. Thus, in the case of a dog which was getting a fixed diet, and was kept constantly in a kennel-cage, so constructed that all the urine passed was collected, I found that on 6 consecutive days the estimations of oxalic acid in the urine were the following :—

Urine of Dog on Fixed Diet. Neubauer's Method used.

Day of Observation.	Quantity of Urine.	Quantity of Oxalic Acid.
1	1250	·025
2	1500	·019
3	1100	·012
4	1500	·053
5	500	·016
6	2280	·072

Also, in the case of the urine of a hospital patient the results were :—

Urine of Hospital Patient. Neubauer's Method.

Day of Observation.	Quantity of Urine.	Quantity of Oxalic Acid.
1	1600	·0089
2	2450	·0094
3	2050	·0091
4	1400	·0219

Here, again, there was no evident cause to account for the increase.

In figures given by other observers, similarly unexplained large results are shown; thus, in Fürbringer's paper (³²), one series of figures is ·0105, ·016, ·055, ·056; another series contains the following, ·011, ·0075, ·0070, ·0060, the first figure of this series being nearly double

the last one. These occasional high results, I believe, are due to some phosphate of lime remaining with the oxalate. This, I believe, because I have repeatedly observed large crystals of phosphate of lime occurring with the second precipitation of the oxalate, *i.e.* from the hydrochloric acid, and also from the fact that phosphate of lime in certain form is extremely difficult to dissolve, requiring a very large volume of cold dilute acetic acid, thus making it a possible and probable source of error.

The theory on which Neubauer's method is based evidently is that all the oxalic acid may be precipitated as calcium oxalate, by the addition of excess of a calcium salt; it does not take into account at all the fact, that in urine there is always an excess of lime present, and that it is no want of lime which leaves the oxalic acid in solution; this being so, adding excess of lime is surely redundant.

4. *Reoch's method.*—The fourth method of estimation previously described is that of Reoch (⁶⁶). He precipitates the oxalates by means of alcohol, letting the urine stand for 24 hours, then examining a drop picked up from the lower part of the urine glass under the microscope, and counting the number of oxalate crystals it contains. From this number he estimates the total number to be found in the specimen of urine, and from the total number, along with an estimated weight of a crystal, calculates the weight of oxalic acid occurring in the specimen of urine. This method is evidently far too inaccurate to give reliable results.

5. *Author's method.*—Consideration of the facts stated above, tending to show the inaccuracy and false premises of Neubauer's method, led me to look for a method of precipitating the oxalic acid out of the urine by the addition of some substance to the urine which does not itself occur there. It was at once evident that a lime salt would not be a precipitant fulfilling these conditions, being always present in excess in urine; alcohol, on the other hand, would be a very suitable precipitant; accordingly, in the method which I have devised, I have adopted Reoch's suggestion of using alcohol: the later stages of the process are adopted from Neubauer's method, with slight variations.

The procedure is as follows:—

Twenty-four hours' urine is collected in a clean vessel, cleanliness being essential, as the less bacterial growth there is the more readily will the urine filter. The filtration is always slow, and if hindered by bacterial growth makes the process almost unworkably long. A well-mixed sample of 500 c.c. is put into a large beaker, and to it is added 150 c.c. of strong spirit, over 90 per cent. alcohol; if the urine is alkaline a little acetic acid should be added, as ammonia is a solvent for oxalate of lime: the beaker is allowed to stand for 48 hours. After this the contents are filtered, great care being taken to remove all sediment adhering to the sides of the beaker; oxalate of lime crystals have a great tendency so to adhere. This is best done by repeated

washing of the beaker, at the same time rubbing it with a glass rod, the end of which is protected by indiarubber tubing. The sediment on the filter paper is then carefully washed with large quantities of water, both hot and cold, and with dilute acetic acid. The filter paper with the sediment is then put into a beaker and soaked with hydrochloric acid—about 5 c.c. being required for this—and then washed with hot water until there is no further acid reaction. The washings are filtered and collected in an evaporating basin, put on a water or sand bath, and reduced to a bulk of about 20 c.c. This is then put into a small beaker, a very little chloride of calcium being added to ensure excess of calcium being present at this stage; the hydrochloric acid is neutralised by adding ammonia, and then the solution is rendered slightly acid by the addition of acetic acid, which favours the crystallisation; strong spirit is then added, to the amount of 50 per cent. of the volume of the fluid, and the beaker with its contents is allowed to stand for 48 hours. The sediment which forms is then collected on an ash free filter paper, washed with water and dilute acetic acid, incinerated in a capsule of known weight, first over a Bunsen burner and afterwards for at least 5 minutes in a strong blowpipe flame; then the capsule with its contents are cooled over sulphuric acid and weighed; the difference between this weight and the weight of the capsule gives the weight of the ash remaining. The ash consists of (1) a trace of ash from the filter paper, which with good paper is so small that it may be disregarded; the papers I used have an ash weighing only about 0.00068 grm.; and (2) quicklime, the action of the long-continued and great heat being to reduce the oxalate of lime first to carbonate of lime and afterwards to quicklime; 1 grm. of quicklime being the equivalent of 1.6 grms. of oxalic acid. The weight of the ash may thus be regarded as the weight of the quicklime obtained, and this multiplied by 1.6 gives the weight of oxalic acid present in the sample of urine analysed.

This process, like that of Neubauer, is a long and tedious one, and takes about 8 or 9 days for its completion, because twice the urine has to stand a considerable time for precipitation, whilst the first process of filtration and washing is always slow. Great care is necessary, especially in removing all traces of sediment from the beakers, and in the weighing, which must be done on a very sensitive balance.

That this process of estimation is a good one is shown by three facts—

1. Oxalate of lime crystals are always recognisable, and found in plenty both in the first and the second precipitation. In this my method differs from that of Neubauer, as, although this is described as occurring, I find that it is the exception, phosphate being often the only recognisable crystal found.

2. The results obtained are much more constant, and the occasional suspiciously large weighings are not met with when care is exercised.

3. Direct observation shows that the oxalate of lime held in solution

by acid sodium phosphate can all be obtained by it. This I have done in the following manner:—Three beakers, A, B, C, were taken. In A there was put some distilled water, in B and C a similar quantity of acid phosphate of soda solution. To each of these were added equal quantities of chloride of calcium solution, and very carefully measured equal quantities of oxalic acid solution; to C there was also added 25 per cent. of strong spirit. The difference in the weights of the precipitates found in A and B shows the amount of oxalate retained in solution by the acid sodium phosphate (this has been previously referred to), while, if this deficiency in B is not repeated in C, it will be evident that the solvent action of the acid sodium phosphate is abolished by the presence of alcohol. My results are seen in the following table:—

Table showing Effect of Alcohol on the Solvent Action of Acid Phosphate of Soda on Oxalate of Lime.

	Beaker A.	Beaker B.	Beaker C.
	Water.	Acid Phosphate Solution.	Acid Phosphate Solution—Alcohol.
1	·0096	·0066	·0096
2	·0246	·0280	·0243
3	·0243	·0227	·0240
4	·0304	·0284	·0308
Total	·0889	·0807	·0887

In these observations the precipitate was, as in the method of analysis described, reduced to quicklime, and the equivalent of oxalic acid calculated from that, the figures expressing the amount of oxalic acid so calculated. These results are so accurate that little, if any, doubt can remain as to the fact that oxalate of lime can be precipitated from solution in acid phosphate of soda solution, by the action of alcohol.

Earlier in this paper I have referred to the possibility of there being present in urine other solvents for oxalate. What these are, should they exist, is not known; and, consequently, direct observation is at present impossible. However, the first two facts I have mentioned in favour of the accuracy of my method tends to show that, whatever they are, their action is overcome by that of alcohol.

In using this method, I have never seen the large, almost insoluble, crystals of phosphate of lime already spoken of as met with when using Neubauer's method; what phosphate there was precipitated occurring as the readily soluble acicular crystals.

To compare the results obtained by this method with those obtained by Neubauer's method, I have estimated the total oxalic acid found in seven different urines, by both methods, and it will be observed that in only two instances are the results similar; while in the rest, with the exception of one, the result by my method is the larger. In the one exception the result is suspiciously high for an estimation by Neubauer's method. The figures show the calculated amount of oxalic acid occurring in 24 hours' urine, and are as stated in the following table:—

Table showing Estimations of Oxalic Acid in total daily Urine, made by Neubauer's and Author's Methods.

	Neubauer's Method.	Author's Method.
1	·0069	·0136
2	·0035	·0035
3	·0116	·0197
4	·0144	·0144
5	·0132	·0366
6	·0083	·0319
7	·0230	·0070

In considering the accuracy of these methods, it must be remembered how small the quantity of oxalic acid in urine is, and how apparent error of observation must be, and, taking into account the complex and changing composition of urine, it is evident that these errors of observation are very liable to occur. Thoroughly reliable results are only to be got by averaging a large number of observations, but the tedious nature of the analyses and the amount of labour they entail prevent any extensive series of observations.

All the estimations in this paper, except a few specially named, were made by my new method. It was only after the adoption of this method that I was able to arrive at satisfactory conclusions, for when using Neubauer's method, as I did in the earlier part of my research, and with which I made a very long series of observations—upwards of a hundred—the results were so uncertain and so contradictory that I was unable to formulate any conclusions.

E. The Amount of Oxalic Acid secreted daily in the Urine.

This has already been estimated by all the four methods I have mentioned, but as the results obtained by the method of Lehmann⁽⁵²⁾, Schultzen⁽⁷⁸⁾, and Reoch⁽⁶⁹⁾ are in no way to be depended upon, it would be superfluous to quote them.

By means of Neubauer's method very careful observations were made by Fürbringer⁽³²⁾, and his conclusions are those generally accepted. He states that while oxalic acid is probably a constant con-

stituent of urine, the daily amount is small, being something less than .020 gm. He does not commit himself, however, as to an average quantity. But taking the figures in his published results, using only those prior to experimental administration of drugs, I calculated that the average he obtained was about .0055 gm.

My own results with Neubauer's method are higher than this, for I find that out of 24 such observations, 12, *i.e.* 50 per cent., are over .01, and the average of the 24, .013; of Fürbringer's observations only 14 per cent. were over .01. In making these comparisons it must be remembered that the usual diet in this country is very different from that in Germany.

The results obtained with my method are, as I have already stated when comparing the two methods, higher than those obtained with Neubauer's method, but to supplement the short table I have previously given I annex a table showing the result obtained from 28 such observations:—

Table showing the Amount of Oxalic Acid contained in a Series of Twenty-eight Urines, as estimated by the Author's Method.

No.	Amount of Urine.	Specific Gravity.	Total Oxalic Acid.
1	1775 c.c.	1017	.0130 grm.
2	1350 "	1022	.0035 "
3	1400 "	1019	.0197 "
4	2175 "	1015	.0304 "
5	600 "	1020	.0194 "
6	2250 "	1014	.0144 "
7	2600 "	1017	.0366 "
8	1850 "	1022	.0319 "
9	1800 "	1017	.0070 "
10	2000 "	1017	.0332 "
11	2300 "	1012	.0090 "
12	1500 "	1020	.0144 "
13	1850 "	1018	.0047 "
14	1300 "	1016	.0157 "
15	1150 "	1020	.0208 "
16	2000 "	1015	.0102 "
17	1750 "	1020	.0268 "
18	1175 "	1029	.0157 "
19	2250 "	1019	.0201 "
20	1400 "	1021	.0116 "
21	2000 "	1014	.0104 "
22	1050 "	1032	.0134 "
23	1100 "	1026	.0112 "
24	1150 "	1030	.0237 "
25	1350 "	1028	.0044 "
26	1500 "	1023	.0201 "
27	1400 "	1025	.0224 "
28	1400 "	1028	.0160 "

In this table, and also in the one previously given (p. 404), it will be observed that by far the greater number are between .01 and .025, while the average of them all is .0172, which is 30 per cent. more than the average which I obtained with Neubauer's method.

The statement of an average I do not consider here as of much importance, because it is taken from a comparatively limited series, only 35 being included in the two tables, and also because the amounts vary so considerably, and I would rather express my conclusion as to the daily amount of oxalic acid excreted normally as being *a quantity usually varying between .01 and .025 grm., but having an average of about .0172 grm.*

The largest amount I have found in a day's urine was .0659 grm., but this large amount was produced by administering oxalate of potassium.

F. *Source of Oxalic Acid of Urine.*

The source from which the oxalic acid present in the urine is derived may be either the oxalic acid in the food-stuffs, or the metabolism of either the carbohydrates or the proteids. The question has been studied by many experimenters, but so far without positive results. The difficulty of making thoroughly satisfactory estimations, and so being able with confidence to recognise the changes brought about by the various experimental procedures, which have to be used in attempts to elucidate it, accounting to a large extent for the contradictory results which the different observers have obtained.

1. *Is Oxalic Acid derived from the Metabolism of the Tissues?*

I shall first consider its possible metabolic source, and afterwards its dietetic source.

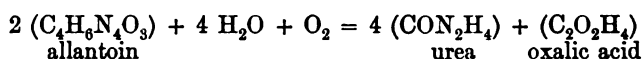
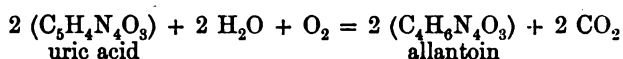
That oxalic acid might possibly be produced by an incomplete oxidation of the carbon compounds is obvious, as oxalic acid, although a very highly-oxidised carbon compound, is not so highly oxidised as carbonic acid. Incomplete oxidation might manifest itself in the excretion of oxalic acid in one of two ways—it might cause the formation of oxalic acid where normally carbonic acid or one of its derivatives is produced, or it might prevent further oxidation of oxalic acid, were it produced normally during the metabolic changes.

a. *Is Oxalic Acid oxidised in the System?*

To allow the existence of the second of these two modes of action, we must allow that oxalic acid can be oxidised in the system. This has never been demonstrated. The observations that have been made tend to prove the contrary. Gaglio (³⁴) investigated this question, whether or not oxalic acid can be oxidised in the body, and found that it could not. He tested it in two ways—first, by artificial-circulation experiments; and, secondly, by injecting oxalate of soda into a hen's crop. In his artificial-circulation experiments he was able to recover from the blood at the end of the experiment as much of the oxalic

submitted to the actions of strong alkali or strong acid, we must conclude that this decomposition does not take place normally in the system.

In the other decomposition of uric acid to which I have referred the intermediate body between uric acid and oxalic acid is allantoin, thus—



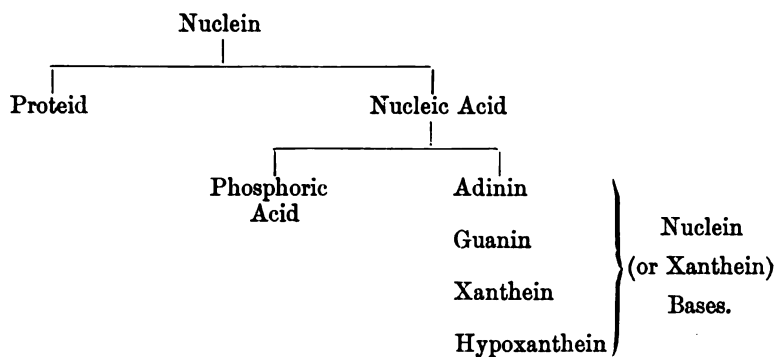
Here, again, although the change is only one of hydration and oxidation, such as one expects would take place in the body, yet it is improbable that it can take place there, as the two stages require very different media for their production, the splitting up of uric acid taking place in the presence of an oxidising agent, such as permanganate of potassium, and the splitting of allantoin taking place under quite different circumstances, namely, digestion with baryta water.

Although neither of these decompositions of uric acid, then, is such as one would expect to take place in metabolism, it does not at all follow that the uric acid is not split up in some other manner, and in doing so give rise to oxalic acid.

But modern observations tend to show that this is not the case, as they tend to prove that the formation of uric acid is quite distinct from the ordinary nitrogenous metabolism where urea is produced, and that uric acid is not, as was at one time supposed, an intermediate body formed during the transition of proteid to urea. The principal work showing this is that of Horbaczewski.¹ He found that there was a production of xanthein bases, if the splenic pulp was kept at the temperature of the body for some time, and so allowed to decompose, and that if this decomposition of splenic pulp took place in presence of abundance of oxygen, then instead of xanthein bases there was a production of uric acid. A similar production of uric acid has been obtained from the decomposition of other tissues rich in leucocytes. Further, he has shown that the formation of uric acid is proportionate to the amount of leucocytosis taking place in the body being greatest during the leucocytosis of digestion; and from this he deduces the fact that uric acid is derived from the breaking up of the leucocytes. He has also noted an increase of uric acid excretion following the administration of nuclein, both to animals and to man, and as nuclein occurs in the nuclei of the leucocytes he infers that the uric acid is produced from the nuclein they contain. Nuclein is capable of being decomposed into a proteid and nucleic acid, the nucleic acid further decomposing into its constituent parts—phosphoric acid and

¹ *Sitzungsbb. d. k. Akad. d. Wissensch.*, Wien, 1891.

xanthein bases, the xanthein bases being oxidised to form uric acid. Von Noorden (⁹²) gives the following table to show these changes :—



The derivation of uric acid from the nuclei of leucocytes is further corroborated by the fact that they are the only cells in the body which occur in sufficient quantity, and which undergo sufficiently rapid change to explain the production of uric acid in the quantity in which it is found in urine; the increase of uric acid found in certain diseases also corroborates this, for in leucocythæmia and in pneumonia, in both of which there is increased leucocytosis, there is also increased uric acid excretion.

Accepting Horbaczewski's theory of uric acid production in the body, and allowing that the uric acid excretion is proportionate to the uric acid so produced, then we must conclude that this uric acid after being so formed is not split up into urea, oxalic, and carbonic acids; further, that if there is a splitting up of uric acid, there must be two sources of its production, the uric acid from one source being split up and from the other not. This is so highly improbable that the natural conclusion is, that there is no splitting up of uric acid at all in the system, and this entails the conclusion that oxalic acid is not formed in the metabolism from uric acid.

Another consideration bearing on this point is, that were uric acid formed as an immediate substance in the metabolism, and split into oxalic acid, carbonic acid, and urea, then the amount of oxalic acid produced would be a very large one, bearing some proportion to the amount of the urea formed, for in both the decompositions of uric acid which I have described there are two molecules of oxalic acid produced from four of urea, or, calculated by weight, there would be three parts oxalic acid for every four parts of urea. Oxalic acid does not act in the system as a neutral body, but is an active poison when present in any quantity. We have no evidence of such poison occurring, and no evidence of its decomposition in the body, and consequently we are again forced to the conclusion that it is not formed from uric acid.

Uric acid, if administered to either a dog or a man, as has been shown by Wohler and Frerichs⁽²⁸⁾, is followed not by an increased excretion of uric acid itself, but by an increased excretion of urea. And this has been used to show that it is under certain circumstances split up in the body, but here the uric acid is subjected to the complex actions of the digestive juices and of the organs of digestion, which uric acid in the system is not. The observations were made on a cat. Large doses of uric acid were given, and it was found that these were followed by a rich sediment of oxalate; or, in other words, that uric acid administered not only gave rise to a formation of urea, but also to an increased production of oxalic acid. They also obtained a similar result by giving it to a man. Wohler and Frerichs used the older method of estimation, and consequently much reliance cannot be placed on this part of their work. Their conclusions, however, have been negatived by those of Fürbringer⁽³²⁾, and Hammerbacher⁽⁴⁰⁾, neither of whom succeeded in getting any increase of oxalic acid after administering uric acid. Fürbringer experimented on men, Hammerbacher on a dog.

Before devising my new method of estimation I made a series of observations on a dog, extending over 27 days, estimating both the uric acid and the oxalic acid excreted every day, but was unable to see any relationship between them. I used Neubauer's method to estimate the oxalic acid, and Ludwig's method to estimate the uric acid. On 6 consecutive days, when the dog was getting a fixed allowance of porridge and milk, my results were as represented in the following table, and these are sufficient to show the very varying proportion between the uric and oxalic acid excreted:—

Table showing Proportion between Uric Acid and Oxalic Acid Excretions.

	Quantity of Urine.	Uric Acid.	Oxalic Acid.	Proportion.
1	900 c.c.	·043	·0207	2 : 1
2	1800 „	·036	·0414	1 : 1
3	1500 „	·033	·0288	1 : 1
4	1750 „	·042	·0268	1·5 : 1
5	600 „	·030	·0083	4 : 1
6	700 „	·028	·0179	1·5 : 1

From these considerations, *I infer that oxalic acid is not oxidised in the process of metabolism, and also that it is not formed by the metabolism of uric acid as has been so frequently stated, and, failing this as the most probable and only suggested metabolic source, that it is not produced in the metabolism at all.*

2. Possible Source of Oxalic Acid from incomplete Combustion of Carbon Compounds.

The other possible way in which incomplete oxidation may bring about the formation of oxalic acid is, as I have already stated, by stopping the combustion of carbon short of the production of carbonic acid. Before allowing that this takes place normally, we are at once met with the difficulty, as pointed out by Esbach⁽²⁵⁾, of understanding how oxidation can have a limited action on such an extremely small portion of the carbon, as, for 140 grms. of carbon excreted as carbonic acid, we have only about $4\frac{1}{2}$ mgrms. excreted as oxalic acid; for $4\frac{1}{2}$ mgrms. is the amount of carbon in 17 mgrms., the average amount of oxalic acid excreted. The amount of carbon excreted as oxalic acid is only about .003 per cent. of the carbon excreted as carbonic acid.

If incomplete oxidation of carbon compounds be the source of production of oxalic acid we should find an increase of the oxalic acid excretion in all conditions which cause dyspnoea. Of this we have as yet no evidence, for the only direct observations made on the subject are open to much suspicion, while clinical observations tend to show that there is no increase.

These direct observations are those made by Reale and Boeri⁽²⁸⁾. They produced dyspnoea, by putting Sayre's apparatus on a dog; and they state that in a dog so treated they found an increase of oxalic acid in the urine, and they compare it to the increased secretion of lactic acid found under similar circumstances by Araki. I am unable fully to criticise Reale's experiments, as I have not been able to see a full account of them. The short note published by them in the *Wiener Medicinische Wochenschrift* merely stating the bare facts, and giving no figures, does not state what precautions were taken against possible fallacy. I cannot, without further proof, accept their experiments as showing that the increase of oxalic acid excreted, were this genuine, was due to mal-oxidation, as dyspnoea produces such complex changes in the system, and may thus act indirectly. Thus dyspnoea increases the movements of the stomach and intestine, possibly also increases the secretion of gastric juice, and in this way might cause an increased absorption of any oxalic acid which was present in the stomach or intestine at the time. Until we know that these and other possible fallacies were excluded, it is impossible to accept Reale's experiments as being conclusive.

The clinical aspect of the question has been carefully studied by Cantani⁽¹⁸⁾. He sums up his conclusion by saying: "Personally, I have never found an abnormal quantity of oxalate of lime in the urine of patients depending on dyspnoea alone, and I must add that in my clinique the urine of nearly all the patients is examined daily, especially so in the case of those suffering from acute or chronic

pleurisy, diffuse chronic bronchitis with emphysema, pneumothorax, severe heart disease, and the like."

A theoretical consideration, also tending to show that mal-oxidation is not the source of the oxalic acid excreted in the urine, is that, if it were so, we should in dyspnoea find a saturation of the tissues with that extremely insoluble salt oxalate of calcium, as lime salts are universally present in the body; such an accumulation of oxalate has never been observed.

That mal-oxidation is not the source of the excreted oxalate is, I consider, conclusively shown by the fact, *that by limiting the diet to milk, and thus stopping the dietetic source, the excretion is entirely prevented.* I have verified this observation several times on different patients and on myself. The only case in which I found oxalic acid with such a diet was my own, and this I accounted for by the duration of the experiment being too short to allow of any oxalic acid which had accumulated in the system, or was remaining in the alimentary canal, being excreted. This limitation of diet, in quality not quantity, can scarcely have action in preventing mal-oxidation. I shall give fuller notes of these experiments when considering the dietetic source with which they are principally connected.

3. *Source of Oxalic Acid from Food-Stuffs.*

All the earlier writers, such as Willis (⁸⁷), Golding Bird (³⁷), Stallard (⁸⁵), Wilson (⁹⁸), and Bartram (⁵), considered that the oxalic acid met with in the urine was derived from food-stuffs. These and other observers associated it with eating such substances as rhubarb, sorrel, and water-cress, or with eating excess of farinaceous food. Stallard advised treating oxaluria by giving a strictly animal diet. The dietetic source was put aside for some years, giving place to the uric acid theory which I have discussed, but has been reintroduced more recently and carefully expounded by several authors, especially by Cantani and Esbach.

That certain food-stuffs are capable of giving rise to a deposition of oxalate in the urine is a fact too well known to require more than mention here. But the wide distribution of oxalate in the vegetable food-stuffs, not only in those substances known to give rise to oxalate of lime deposit, when eaten in excess, but also in many others which are in daily use, and which are generally supposed to be inert in this respect, is a fact which must be considered when searching for a source of the oxalic acid excreted from the body. Esbach gives an elaborate series of estimation. By what method they were made he does not state, but being so complete a list I quote the principal of them, the figures referring to parts per thousand.

Substance.	Oxalic Acid.	Substance.	Oxalic Acid.
Black tea	3·7 per 1000	Green beans . . .	0·2 per 1000
Black tea, 5 minutes' infusion	2·0 „	Tomatoes	0·05 „
Cocoa	4·5 „	Carrots	0·03 „
Chocolate	0·9 „	Celery	0·02 „
Pepper	3·2 „	Green peas	Doubtful
Chicory	0·7 „	Turnip	„
Coffee (<i>mélange d'amateurs</i>)	0·1 „	Asparagus	„
Beans	0·3 „	Cucumbers	„
Potatoes	0·4 „	Mushrooms	„
Lentils	Doubtful	Onions	„
Peas	„	Leeks	„
Rice	„	Endive	0·1 „
Bread	0·047 „	Cress	Traces
Crust	0·13 „	Lettuce	Doubtful
Flours, various	0-0·17 „	Figs, dried	1·3 „
Sorrel	3·6 „	Gooseberries	0·13 „
Spinach	3·2 „	Plums	0·12 „
Rhubarb	2·4 „	Strawberries	0·06 „
Brussel sprouts	0·02 „	Apples	Traces
Cabbage and cauliflower	Doubtful	Pears	Doubtful
Beetroot	0·4 „	Apricots	„
		Peaches	„
		Grapes	„
		Melons	„

Abeles⁽¹⁾, also gives some estimations of oxalic acid in the vegetable food-stuffs. The method he used in making these estimations was the following:—He boiled the substance to be examined in water, filtered and estimated the oxalic acid in the filtrate by Neubauer's method, thus getting an estimation of the soluble oxalate. He then boiled the solid residue in dilute hydrochloric acid, and estimated the oxalic acid taken from the substance in this way, and so obtained an estimation of the insoluble oxalate.

The following are the results of analyses which he gives:—

	Oxalic Acid as—		
	Soluble Oxalate.	Insoluble Oxalate.	Total.
Spinach, dried, 10 grms. . . .	·1671	·1306	·2977
„ 10 „	·1365	·1208	·2573
„ 10 „	·3649	·1478	·5127
„ 10 „	·2726	·2731	·5457

In the two last observations samples of the same spinach were used, the difference being due to distilled water being used in the first and Wiener Hochquelle water in the second estimation.

	Oxalic Acid as—		
	Soluble Oxalate.	Insoluble Oxalate.	Total.
Asparagus dry, 200 grms. . . .	·0048	·0025	·0068
Carrot, dry, 100 grms. . . .	Trace	Trace	Trace
Tomato, dry, 500 grms. . . .	·0099	Trace	·0100
Tea,	·0166	·0268	·0434

These figures are not easily compared with those of Esbach, but in the case of the spinach it may be seen that his results corroborate Esbach's. Thus, spinach contains 90 per cent. water. Abeles, in his four estimations, got in all 1·6134 grms. of oxalic acid from 40 grms. of dried spinach, which is equivalent to 400 grms. ordinary spinach, 1·6 in 400 is equivalent to 4 per thousand, the amount stated by Esbach being 3·2. He also states that tea contains ·0434 oxalic acid in 10 grms., that is 4·3 grms. per thousand, Esbach estimating it at 3·7.

I have analysed tea infusion, flour, and potato, by the method which Abeles used, and my results were:—

Tea infusion,	1·7 per 1000 of tea.
Flour,	1·1 per 1000.
Potatoes,	·8 „

It will be noted that my estimation of the oxalic acid in tea infusion corresponds nearly to that of Esbach; my estimations of that in flour and potato are considerably higher than Esbach's.

As a summary of these tables, it may be stated that oxalic acid occurs in very considerable quantities in many vegetable food-stuffs, which are in daily use; and that in some of these, notably tea, coffee, pepper, sorrel, spinach, and rhubarb, it occurs in relatively large quantities.

When oxalic acid or oxalate is swallowed, its solution or precipitation in the stomach depends on there being excess of free acid, and on whether or not there is excess of lime. That some acids can prevent the precipitation of oxalate of lime is a well-known fact in chemistry. The mineral acids do this, and also some organic acids, one of which is lactic acid. In the stomach we have both free hydrochloric acid and lactic acid; lactic acid being present during the earlier stage of digestion, hydrochloric acid when full digestion is advanced. Both of these are capable of keeping oxalic acid in solution, and thus tend to help its absorption.

Practically, lime is always in the stomach during digestion, for not only is it a constituent of both vegetable and animal foods, but it also occurs in drinking water. The lime, of course, tends to precipitate

oxalic acid, and so tends to prevent its absorption. Consequently, during the gastric digestion of an ordinary mixed meal we have in the stomach oxalic acid, which may or may not be, in a soluble form, acids favouring absorption and lime hindering it. This oxalic acid is, I believe, the source of the oxalic acid secreted in urine. To these two influencing factors I consider that the changing quantity of the oxalic excretion is due. I shall consider these points in detail.

Absorption of Oxalic Acid.

That oxalic acid or soluble oxalate can, at all events in part, be absorbed, is demonstrated by the fact that the administration of oxalic acid or oxalate is followed by an increase of the oxalic acid in the urine, and that eating food-stuffs rich in oxalates has the same effect. Earlier observers recognised this merely in so far as a rich sediment of oxalate of lime was produced. Now, to have a rich sediment of oxalate we must, as I have shown earlier in the paper, have a comparatively high percentage of it in the urine, and this occurring repeatedly surely indicates an excess being secreted. Duckworth (²³), who repeated some experiments made by Leared,¹ showed that by administering oxalic acid a copious deposition of oxalate in the urine was produced. More recently chemical analyses have been employed, and in the observations of Bucheim (¹⁷), of Marfori (⁵⁶), and of Abeles (¹), administration of oxalic acid, either as a drug or in a food rich in oxalate, was followed by an increased excretion—not a large increase, but a decided one. Esbach (²⁵), by swallowing 5 grms. of oxalic acid, increased the amount of oxalic acid in his urine to 181 grms., which is the largest amount ever demonstrated by Neubauer's method.

I have satisfied myself of this increase by making two observations on patients; in both cases there was a marked increase of the oxalic acid excretion, following the administration of potassium oxalate. The following tables show the result of these observations, the estimations being done by my new method:—

PATIENT 1.—*Disease—Early Disseminated Sclerosis. Diet—Hospital Convalescent Diet.*

Day.	Oxalic Acid Excreted.	Drug Given.
1	·0116 grms.	...
2	·0107 "	...
3	·0348 "	·6 gm. potas. oxalate.
4	·0338 "	"
5	·0104 "	...
6	·0090 "	...

¹ *Med. Times and Gaz.*, London, 1867, vol. i. p. 219.

PATIENT 2.—*Disease—Early Disseminated Sclerosis. Diet—Hospital Convalescent Diet.*

Day.	Oxalic Acid Excreted	Drug Given.
1	·0157 grms.	...
2	·0069 "	...
3	·0280 "	·6 grm. potas. oxalate.
4	·0238 "	"
5	·0240 "	"
6	·0201 "	...
7	·0018 "	...
8	·0134 "	...

These experiments, along with previous observations of others, are sufficient to show that oxalic acid can be absorbed from the contents of the stomach, and, after absorption, excreted in the urine.

Is Oxalic Acid, absorbed, the only Source of the Oxalic Acid of the Urine?

To investigate whether or not oxalic acid absorbed from the food stuffs is the only source of oxalic acid of the urine, I have made observations on patients whose diet was restricted to one which was free from oxalic acid, and have noted the effect of that on the excretion of oxalic acid. Such a diet we have in milk, and in the cases on which I made these observations the diet was absolutely restricted to milk. I have examined the urines of seven such cases, using the alcohol test, and, with one exception, was unable to find any trace of oxalic acid in the urine. This exception was during an observation I made on myself, which, however was not continued long enough to give definite results. Putting this exception aside, as it cannot be considered a fair test, I have satisfied myself *that on a pure milk diet, sufficiently long continued to allow of any oxalate which may have been in the system being excreted, the excretion of oxalic acid in the urine ceases.* Also, these cases getting pure milk diet yielded the only urines (and I have examined some hundreds altogether) in which I was unable to recognise oxalic acid.

Esbach (²⁵) states that he stopped the excretion entirely by restricting the diet to bread without crust (*vide* Esbach's tables, p. 413) and meat. That this diet very markedly diminished it I have no doubt, but without using the alcohol test, which presumably he did not use, I cannot accept complete stoppage as demonstrated.

The experiments with milk diet I have carried a stage further in three cases, as in these, after stopping the excretion with milk diet, I was able to start it again by giving some tea, which, by reference to the foregoing tables, will be seen to contain oxalic acid. In all three cases, tea established an abundant excretion of oxalic acid.

The following show details of my observations on them.

OBSERVATION 1.—*Woman. Disease—Gastric Ulcer.*

Day.	Oxalate in Urine.	Diet.
1	Absent.	Milk.
2	„	„
3	„	„
4	Present.	Milk and tea.

OBSERVATION 2.—*Woman. Disease—Gastric Ulcer.*

Day.	Oxalate in Urine.	Diet.
1	Absent.	Milk.
2	A trace present.	„
3	Absent.	„
4	„	„
5	Present.	Milk and tea.

OBSERVATION 3.—*Made on the Author.*

First day.—Full mixed diet. Weight, 11 st. 4 lb.; urine, 1500 c.c.; specific gravity, 1026; urinary solid total, 107 grms. *No sediment of oxalates*, but after alcohol test copious sediment of oxalate.

Second day.—Diet, 1 gallon milk and 4 oz. cream. Weight, 11 st. 3 lb.; urine, 3400 c.c.; specific gravity, 1015; urinary solids, 102. *No oxalates in sediment*; none with alcohol test, and none after evaporation to half volume with alcohol test.

Third day.—Diet as on second day. Urine, 3000 c.c.; specific gravity, 1015; urinary solids, 90. Sediment contained no oxalates, but with alcohol test *a few were found*, and also when applying this test to a concentrated specimen.

Fourth day.—Diet, milk, 7 pints., cream, 4 oz., and three cups of strong tea. Weight, 11 st. 2 lb.; urine, 3200 c.c.; specific gravity, 1014; total urinary solids, 93 grms. Sediment contained no oxalates, but with alcohol test showed *very considerable quantity*, as was also seen by applying this test to a concentrated specimen.

Fifth day.—Full mixed diet. A specimen of urine showed with alcohol test *abundance of oxalates*.

Note.—It was not till the fifth day that the fæces got the typical appearance seen with a milk diet.

It will be observed that in both the second experiment and the third, the one carried on on myself, that on the second day of milk diet there was found some oxalate. This I attribute to the excretion of

some oxalic acid not yet absorbed from the alimentary canal. In the second experiment neither the third nor fourth day's urine contained any. In the third experiment, most unfortunately, the pure milk diet was not longer continued. Had I anticipated this result I should certainly have continued it, but not anticipating it, and finding milk a poor diet on which to do a day's work in frosty weather, I stopped it at what I considered the earliest opportunity to show the action of the milk. The experiment, undoubtedly, showed a great diminution of the oxalate excretion and an increase of it when tea was added to the diet.

Abeles has argued and concluded that it is not from this dietetic source that the oxalic acid, found in the urine of an oxaluric patient, is derived; as there is, he says, not sufficient oxalic acid in an ordinary diet to produce what oxalate is found in the urine. I cannot agree with this conclusion, for the amount in the urine is always small, .017 grms. being the average. Now, surely, if there is any recognisable quantity of oxalic acid in the food-stuffs this small amount will be taken. A man eats about 500 grms. of vegetable food in the day, mostly in the form of bread and potato; according to Esbach's figures this alone will contain .02, and this makes no allowance for the additional amount of oxalate which is to be found in the crust. If the man were a tea-drinker, as the majority in this country are, he would take the infusion of about 36 grms. of tea, which would contain .01 (or .02 according to Abeles' figure), and thus from these two articles of diet alone he gets more than the equivalent of the total amount excreted, without making any allowance for his taking other articles rich in oxalic acid. My own opinion is that probably Esbach's figures are too low, but having made so few estimations of the food-stuffs I cannot criticise them fully.

Influence of Factors on Absorption of Oxalic Acid.

If the oxalic acid in the food is the source from which the excreted oxalic acid is derived, then, as I have before stated, it would be expected that its absorption and subsequent excretion would be influenced by the amount of acid, and by the amount of lime present along with it in the stomach. It is to the influence of the acids on it that I have specially devoted observation, and from the results of the following five experiments, it is evident that excess of hydrochloric acid *does increase the absorption and subsequent excretion of oxalic acid.*

Hydrochloric Acid.

The patients on whom these observations were made were none of them suffering from any form of dyspepsia, and were getting no drug other than the hydrochloric acid.

OBSERVATION 1.—*Patient in Infirmary. Diet mixed.*

Day.	Quantity of Urine.	Oxalic Acid Excreted.	Drugs.
1	1850 c.c.	·0047	...
2	1300 „	·0157	...
3	1350 „	·0127	Hydrochloric acid.
4	2200 „	·0268	„
5	2450 „	·0556	„
6	1150 „	·0208	...
7	2000 „	·0102	...

Note.—When hydrochloric acid was given, it was given as acidum hydrochloricum dilutum B.P.; 60 minims half-hour after food, and repeated 1 hour after food; in all, 360 minims per diem.

OBSERVATION 2.—*Patient in Infirmary. Diet mixed.*

Day.	Quantity of Urine.	Oxalic Acid Excreted.	Drugs.
1	1350 c.c.	·0044	...
2	1500 „	·0201	...
3	1150 „	·0228	Hydrochloric acid.
4	1750 „	·0246	„
5	1550 „	·0297	„
6	1400 „	·0224	...
7	1400 „	·0160	...

OBSERVATION 3.—*Patient in Infirmary. Diet mixed.*

Day.	Quantity of Urine.	Oxalic Acid Excreted.	Drugs.
1	1900 c.c.	·0312	...
2	2050 „	·0272	...
3	1850 „	·0323	Hydrochloric acid.
4	2150 „	·0659	„

OBSERVATION 4.—*Patient in Infirmary. Diet mixed.*

Day.	Quantity of Urine.	Oxalic Acid Excreted.	Drugs.
1	1700 c.c.	·0324	...
2	2200 „	·0128	...
3	2000 „	·0460	Hydrochloric acid.
4	2000 „	·0250	„

Note.—In Observations 2, 3, and 4 the hydrochloric acid was given in the same manner as in Observation 1.

The last experiment made on myself was the most thoroughly testing one, as during it I put myself on a fixed diet, a thing I was unable to do satisfactorily with the hospital patients. My diet consisted of:—

Breakfast.—Porridge and milk, two eggs, toast and tea.

Lunch.—Lean cold roast beef, bread and butter.

Dinner.—Soup—one packet of Edward's dessicated tomato soup, cooked in accordance with instructions on packet; minced beef, with boiled rice; bread, butter, cheese, and beer; the quantities of all these being carefully weighed, and as exactly as possible the same amount was used daily.

OBSERVATION 5.—*The Author. Diet as stated above.*

Day.	Quantity of Urine.	Oxalic Acid Excreted.	Drugs.
1	2150 c.c.	·0230	...
2	1650 „	·0216	...
3	2800 „	·0280	Hydrochloric acid.
4	3150 „	·0385	„
5	2600 „	·0466	„
6	2600 „	·0433	...
7	2100 „	·0295	...

Note.—Hydrochloric acid given as in other observations.

In this last experiment and also in the first two it will be observed that the oxalic acid excretion did not immediately fall when the hydrochloric acid was stopped. This, I consider, is due to a certain amount of storage of oxalic acid having taken place in the body, and is perfectly in harmony with the theory that the increase was due to an increased absorption.

The following table shows the result of these experiments at a glance. In it I have averaged the amount of oxalic acid excreted per

diem, without and with the administration of the hydrochloric acid. In doing so, I have excluded the day immediately following the stoppage of the acid :—

Table showing Average of above Observations.

Observation Number.	Average Oxalic Acid Excreted.	
	Without Administration of Acid.	With Administration of Acid.
1	·0102	·0317
2	·0122	·0215
3	·0292	·0491
4	·0226	·0355
5	·0280	·0379
Average	·0204	·0351

It is evident from this table that the administration of hydrochloric acid, in the large doses which were used, is followed by an increased excretion of oxalic acid. It has long been held, and it has recently been proved, by Lockhart Gillespie,¹ that hydrochloric acid in these large doses causes an increase of the gastric acidity, and it is to this increased gastric acidity that I consider the increased absorption and excretion of oxalic acid is due.

Lactic Acid.

I have made two observations on the effect of lactic acid administration on the oxalic acid excretion, and in both cases have found a decided increase of the oxalic acid excreted. These were both made on hospital patients, the same as were used for the hydrochloric acid observations 3 and 4. My results were :—

OBSERVATION 1.—Patient in Infirmary. Diet mixed.

Day.	Oxalic Acid Excreted.	Drugs.
1	·0110	...
2	·0321	...
3	·0239	Lactic acid.
4	·0382	„

Note.—Lactic acid, given as acidum lacticum dilutum B.P.; 60 minims half-hour before, and repeated 1 hour after, food; in all, 360 minims per diem.

¹ *Trans. Med.-Chir. Soc. Edin.*, vol. xiii.

OBSERVATION 2.

Day.	Oxalic Acid Excreted.	Drugs.
1	·0239	...
2	·0119	...
3	·0201	Lactic acid.
4	·0200	„

Note.—Lactic acid given as in Observation 1.

Table showing Averages of these two Observations.

Observation Number.	Average Excretion of Oxalic Acid.	
	Without Administration of Lactic Acid.	With Administration of Lactic Acid.
1	·0210	·0310
2	·0179	·0200
Average	·0194	·0255

Influence of Lime Salts.

I have made only one observation on this point, and in it I found a marked diminution of the amount of oxalic acid excreted.

OBSERVATION 1.—*Oxalic Acid estimated by Neubauer's Method.*
Patient in Infirmary. Mixed Diet.

Day.	Oxalic Excreted.	Drugs.
1	·0089	...
2	·0094	...
3	·0091	...
4	·0219	...
5	·0057	Calcium chloride.
6	·0078	„
7	·0069	„

Note.—Calcium chloride given in 15-gr. doses thrice daily after food.

These observations made on the factors influencing the excretion of oxalic acid not only show that they can influence it, but also tend to show that the theory that the oxalic acid, which is excreted in urine, is derived from oxalic acid in the food-stuffs is correct, because these factors are such as act upon absorption of oxalic acid, and, influencing absorption, they also influence the amount excreted. It is of great importance to know that these factors, and especially the amount of acid in the stomach, do influence the excretion of oxalic acid when considering the etiology of the increased excretion met with in disease.

APPLICATION OF THESE INVESTIGATIONS TO OXALURIA.

There remains for consideration the bearing of these observations and theories on the pathological condition known as oxaluria. By oxaluria I mean the condition referred to in Begbie's paper, not a case where there is a deposit of oxalate of lime in the urine, but where, in addition to this deposition, there exists a well-known combination of symptoms. That such cases do exist, I have not the slightest doubt; I have seen typical ones and treated them, but the fact of their rarity in the hospital wards shows that they are not nearly so common as some would lead us to suppose.

The symptoms of oxaluria are of four kinds—(1) altered condition of urine, (2) dyspepsia, (3) pains, (4) nervous symptoms; and to ascertain where the morbid condition really exists it is necessary to examine each of these separately.

1. *The condition of urine.*—In the recorded cases of oxaluria there is only one condition which is always associated with it, and that is, of course, a sediment of oxalate of calcium. This symptom has been considered so important that the morbid condition has been named from it. That this symptom by itself is certainly not sufficient to enable one to diagnose the condition referred to by the name oxaluria, is evident from the frequent occurrence of oxalates in the urine of healthy people and of those suffering from absolutely distinct morbid conditions. As previously stated, my own observations enable me to put down the deposition of oxalic acid as occurring in one urine out of three, and this estimate is exceeded by figures which have been given by others.

The significance of a deposit of oxalates I have already discussed, and believe that it does indicate an increased excretion, and consequently, in oxaluria, that there is an increase of the oxalate excreted. This increased excretion is necessarily derived from an increase of the oxalic acid normally in the system, which I have concluded is entirely derived from oxalic acid contained in food-stuff, or from some other source which in health does not produce oxalic acid. From which of these two sources it occurs we have no

indication from the condition of the urine, for all that has been observed about it is that it usually, not always, has a fairly high specific gravity, 1020 up to 1025 being what is generally stated, and that it usually has a rather low degree of acidity, so before discussing the source it is necessary to criticise the other symptoms which are constantly present.

2. *Pains*.—The principal pains to consider are headache, pains in the region of the stomach, and pains in the lumbar region. The first two of these fall to be discussed under nervous symptoms and dyspepsia respectively. The explanation of pain in the lumbar region commonly given, and with which I perfectly agree, is that it is due to irritation set up by the presence of crystals of oxalate of lime in the pelvis of the kidney and the ureter; the crystals, though very small, have very sharp angles, and may cause this irritation mechanically. That the deposition of oxalate of lime can take place in the pelvis of the kidney, or even in the kidney tubules, is evident from the fact that they are found inside urinary casts in some cases recovering from cholera, where there is a suppression of the filtration of water from the glomeruli, probably due to the fall of blood pressure which occurs, and where, in consequence, one would expect to find the less soluble constituent of the urine deposited inside the uriniferous tubules. This pain in the lumbar region, then, is to be explained by the presence of oxalate crystals in the urine which passes through the pelvis of the kidney and the ureter, but does not give any further indication of the pathology of the condition.

3. *Dyspepsia*.—This is a constant symptom in oxaluria, and is, I believe, the principal one, for to the presence of dyspepsia one can refer all the other symptoms.

There are three reasons which make me consider dyspepsia as the essential part of the pathological condition known as oxaluria. These are—(1) experimental evidence, (2) similarity of symptoms between oxaluria and acid dyspepsia, and (3) similar treatment alleviates both conditions.

(1) *Experimental evidence*.—By this I refer to my observations, showing that by administering large doses of acid, and so artificially producing a condition similar to hyperacid dyspepsia, one can produce an increased excretion of oxalic acid in the urine. In my experiments the effect of the acid was to increase the oxalic acid excretion by an average amount of nearly 75 per cent.

(2) *Similarity of symptoms between oxaluria and hyperacid dyspepsia*.—Cantani, who gives the fullest description of oxaluria, describes the digestive symptoms as “indigestion, oppression in the epigastrium, torpor, and dilation of the stomach, dyspepsia, acidity, sluggish action of the bowels, constipation, flatulence, and colic.” Hayem,¹ in his description of hyperchlorhydrie, mentions all these symptoms as frequent

¹ “Leçons de Thérapeutique.”

in that condition, and what points more conclusively to the similarity of the two forms of dyspepsia—the oxaluric and the hyperacid—proceeds to describe the typical pains and nervous symptoms met with in oxaluria. Thus, “Beaucoup de malades se plaignent de douleurs dans le côtés et dans le dos ou bien encore dans les flancs et jusque dans la partie inférieure des lombes. Ces derniers phénomènes sont mêmes assez souvent très pénibles.” Also, “Les symptômes nerveux sont très fréquents. Les troubles nerveux revêtent assez souvent la forme neurasthénique, avec prédominance des signes cérébraux: les malades dorment mal et irrégulièrement, ils sont impressionnables, très susceptibles; tout leur est agacement, aussi sont-ils très désagréables pour leur entourage. Il est même vraisemblable que l’hyperpepsie prédispose aux psychoses. Le suicide n’est pas très rare chez les hyperpeptiques.” Ewald¹ fully recognises the close relationship between hyperacidity and nervous conditions; in fact, considers it to be a sensory neurosis of the stomach. He describes how it can lead on to atony and dilation of the stomach, and so give rise to many symptoms, including flatulence. He also remarks on the impossibility of accurately diagnosing it without chemically analysing the gastric contents. One frequent symptom of the dyspepsia of oxaluria, which might be supposed to negative this theory of the similarity between it and acid dyspepsia, is flatulence, but Hayem, who has made such very careful analyses of gastric contents, states that it is by no means uncommon in acid dyspepsia; he noted flatulence as a symptom in 20 out of 58 cases of hyperacidity examined by him.

Whether hyperacid dyspepsia is really a derangement of the stomach as taught by Hayem, or a neurosis as taught by Ewald, is immaterial in discussing its similarity to oxaluric dyspepsia, for whatever the true pathology is, it will equally explain the same symptoms occurring in the two conditions.

(3) *Similar treatment used in oxaluria and in hyperacid dyspepsia.*—The third reason which indicates the similarity between the hyperacid dyspepsia and oxaluria is, that they are both benefited by the same treatment, for the ordinary treatments adopted, which also relieve the symptoms of oxaluria, are either to administer acid before food, or alkali after it; both of these being capable of reducing the acidity of the stomach contents.

(4) *Nervous symptoms.*—The characteristic nervous symptoms met with are headache, loss of sleep, irritability, and loss of energy, and are, as is evident from the description of the nervous symptoms met with in hyperacidity which I have quoted from Hayem’s work, such as are expected to accompany hyperacid dyspepsia.

It might be argued that the nervous symptoms are to be explained by there being present an excess of oxalic acid in the system, oxalic

¹ “Diseases of the Stomach.”

acid being a nerve poison, as is shown by the fact that when taken in great excess it produces torpor, convulsions, and tingling sensations.¹ But to produce these effects the quantity must be very large, very many times greater than the amount probably present in the system of an oxaluric patient; this is well shown by the result of an experiment which Esbach (²⁵) performed on himself; he swallowed 2·039 grms. of oxalic acid, and excreted in the following 24 hours ·181, showing that a quantity of oxalic acid, immensely greater than in any previous observations made, was passed through his system, and yet no nervous phenomena occurred.

That the oxalic acid passed by an oxaluric patient is derived from the food-stuffs is shown by the successful treatment of the condition recorded by Cantani (¹⁸), who put his patients on a purely animal diet, and was so able to prevent the deposition of oxalate in the urine and relieve the pains in the lumbar regions. His observations do not conclusively show that the excretion was entirely arrested, as he made neither quantitative tests nor any qualitative tests other than direct observation, but the repeated success recorded by him shows at all events a very great diminution of the oxalate excreted.

This source of the oxalate excreted is by no means a new theory; it was written about by the earlier authors, and fifty years ago the condition was treated on this hypothesis by Stallard (⁸⁵), but that it is the correct theory is, I consider, shown by the observations of others on oxaluric patients, and by my own observations on the physiological excretion of oxalic acid.

From this consideration of the source of the oxalic acid excreted, and from the fact shown by the critical discussion of the symptoms, that acid dyspepsia is the essential condition, itself capable of giving rise to the same series of symptoms as oxaluria, and being relieved by the same modes of treatment, I conclude that oxaluria is no special pathological condition, but must be regarded as a form of hyperacid dyspepsia, and should be recognised and treated as such.

I append a list of conclusions extracted from this paper, and also a list of works referring to my subject.

CONCLUSIONS.

1. That oxalic acid is a constant constituent in the urine of men eating an ordinary mixed diet.
2. That in urine there is always present an excess of a calcium salt which tends to precipitate oxalic acid, but this precipitation is prevented in the majority of urines by acid sodium phosphate, and possibly also by other substances.
3. That this precipitation is most liable to occur when the percentage of oxalic acid in the urine is comparatively high.

¹ Krohl, *Arch. d. Pharmakol. Inst. zu Dorpat*, Stuttgart. 1891.

4. That precipitation of oxalate of calcium occurs in about one urine out of every three.
5. That oxalate of lime is recognisable only as octohedral crystals.
6. That the methods previously employed are faulty.
7. That the daily amount of oxalic acid excreted in the urine is small, usually varying between .010 and .0250 grms., and averaging about .017 grms.
8. That alcohol is an efficient precipitant for oxalate, and may be used both in quantitative and qualitative analysis.
9. That oxalic acid is not produced in the metabolism, either from the nitrogenous metabolism or from imperfect oxidation of carbon compounds.
10. That oxalic acid can be and is absorbed from the alimentary canal.
11. That oxalic acid so absorbed is not oxidised in the body, but is excreted as such.
12. That the amount absorbed depends on the amount taken in the food or in drugs, and on various conditions which may aid the absorption, notably being increased by the amount of acid in the stomach.
13. That oxaluria is no special pathological condition, but is essentially a hyperacid dyspepsia, and that all its symptoms can be referred to the existence of acid dyspepsia.

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ACETONURIA AND GENERAL ANÆSTHESIA.

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ACETONURIA has been found in association with many conditions, *e.g.* diabetes mellitus, fevers, starvation, some forms of cancer, auto-intoxication, and insanity. In addition to these generally accepted causes, von Jaksch¹ states that acetone is a normal constituent of the urine, and Becker² that acetonuria follows anæsthesia in at least two-thirds of the cases, irrespective of the nature of the anæsthetic or the duration of the anæsthesia. In investigating the accuracy of Becker's statement the following method was adopted. The urine was examined before operation, and the examination continued daily until the urine returned to the condition (as regards acetone) in which it was found before operation. A fair amount of urine was always distilled before acetone was said to be absent. Legal's nitro-prusside test was used throughout, as Becker's statement is that acetone can, by this test, be detected in the urine directly in two-thirds of the cases.

In the earlier cases of my series Lieben's iodoform test and Reynold's mercuric oxide test were used as confirmatory tests, but afterwards the nitro-prusside reaction and the characteristic smell were relied upon as satisfactory proofs of the presence of acetone.

In the table, C = chloroform, E = ether, and "Acetone*" signifies that acetone was only detected by distillation.

The result of my investigation has been to confirm Becker's statement, the correspondence between his results and my own being curiously exact. In 188 cases Becker found acetone in the urine by Legal's test 125 times (66 per cent.), in my 25 cases I found it in 16 (64 per cent.). In the remaining 9 cases I found acetone by distillation. Apparently we may conclude that acetonuria invariably occurs to a greater or less extent after general anæsthesia.

In the cases I have examined chloroform, ether, or chloroform and

¹ "Klin. Diagnostik."

² *Deutsche med. Wchnschr.*, Leipzig, No. 19, 1895.

No.	Name, Age, and Disease.	Before Operation.	Nature and Amount of Anæsthetic.	Duration of Anæsthesia.	Days after Operation.										Remarks.
					1	2	3	4	5	6	7	8	9	10	
1	Susan P., 45; Carcin. mammae.	No acetone.	C. 3lj.; E. 3lj.	½ hour.	No acetone.	Acetone.*	?	No acetone.							No temperature.
2	James L., 40; Varicose veins.	"	C. 3lj.; E. 3lj.	"	"	"	Acetone.*	"	No acetone.						No temperature.
3	John H., 36.	"	C. 3lj.; E. 3lj.	?	Acetone.*	"	"	Acetone.*	"						No temperature.
4	John L., 86; Loose cart. in knee-joint.	"	C. 3lv.	?	"	"	"	No acetone.							Temperature, 102° on 2nd day.
5	Geo. P., 18; Tubercular testis.	"	C. 3lv.	?	Acetone.*	Acetone.*	"	Acetone.*	"						Irregular temperature.
6	Wm. D., 85; Fistula in ano.	"	C. 3lv.	?	Acetone.	Acetone.	No acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.		Temperature, 102° on 2nd day.
7	Wm. T., 11; Caries of os calcis	Acetone.*	C. 3lv.	40 mins.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.		No temp. Rectal feeding for 8 days.
8	Thos. FitzH., 24; Fibrous stricture of pylorus.	No acetone.	C. 3lv.; E. 3vi.	1½ hours	No acetone.	"	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.	Acetone.		Brand's essence and chicken-broth for 8 days; milk, 8th day. Temperature on 1st day, 101°-4.
9	Jas. L., 54; Fistula in ano.	"	C. 3lj.; E. 3lj.	30 mins.	"	Acetone.	Acetone.*	?	Acetone.*	No acetone.					No temp. Vomiting, 1st day. No temperature.
10	Eliz. B., 80; Bursa patellæ.	Acetone.	C. 3ss.	30 mins.	Acetone.	Acetone (copious).	Acetone.	Acetone.	Acetone.	Acetone.*	Acetone.*				Temp., 100°-4 on 1st, 5th, and 7th days. No temperature.
11	John E., 38; Femoral aneur.	Acetone.*	C. 3ss.; E. 3ij.	85 mins.	"	Acetone.	"	"	"	Acetone.*	Acetone.*				No temperature.
12	John D., 49; Pyonephrosis.	No acetone.	C. 3ij.	?	Acetone.*	Acetone.*	"	Acetone.*	Acetone.*	Acetone.*	Acetone.*	?	No acetone.		Temp., 100°-4 on 1st, 5th, and 7th days. No temperature.
13	Alfred O., 17; Varicocele.	"	C. 3lv.	15 mins.	"	"	"	Acetone.	Acetone.	"	"	No acetone.			No temperature.
14	Margt. A., 17; Tuberc. glands.	"	C. 3ij.	12 mins.	No acetone.	"	Acetone.*	Acetone.*	No acetone.	"	"				No temperature.
15	Lily M., 26; Adeno-fibroma.	Not examined	C. 3ij.; E. 3ij.	30 mins.	Acetone.	No acetone.									No temperature.
16	Sarah N., 24; "	"	C. 3lj.	12 mins.	"	Acetone.*	Acetone.*								No temperature.
17	Mrs. B., 56; Carcinoma mam.	"	C. 3lv.	25 mins.	"	Acetone.*	Acetone.*								No temperature.
18	Ann M., 87; Dislocation of jaw.	Acetone.*	C. 3ss.; E. some	15 mins.	"	Acetone.	Acetone.*	"	Is charged.						Alcoholic subject.
19	Harold R., 18; Morbus coxæ.	No acetone.	C. 3ss.	40 mins.	No acetone.	Acetone.*	"	Acetone.	Acetone.*	?	No acetone.				Temperature, 100°.
20	Lancelot A.; Hernia.	"	C. 3vi.	25 mins.	Acetone.	"	"	Acetone.	Acetone.*						Temperature, 100° on 1st day.
21	Henry H.; Caries skull.	Acetone.*	C. 3lj.; E. 3ix.	1 hour.	Acetone.*	"	No acetone.	"	Acetone.*						Temp., 100°-8 on 1st day, 101°-2 on 2nd day.
22	John C., 21; Varicocele.	"	C. 3ix.	30 mins.	Acetone.	Acetone.	Acetone.	"	Acetone.*						Temperature, 101°-2 on 2nd day.
23	Sydney S., 30; Necrosis of ribs.	"	C. 3lv.	20 mins.	"	"	"	Acetone.*	"						No temperature.
24	Susan M'A., 17; Necrosis of phalanges.	"	C. 3ij.; E. 3ij.	20 mins.	"	"	"	Acetone.	Acetone.						Temperature, 101°-2 on 2nd day.
25	Violet S., 43; Hemorrhoids.	Acetone.	C. 3ss.; E. 3ss.	25 mins.	Acetone.	Acetone.*	No acetone.	Acetone.	"						No temperature.

ether were the anæsthetics used in Becker's cases; and in addition to these, bromæther was employed; consequently, the occurrence of acetonuria cannot be ascribed to the nature of the anæsthetic.

Age, sex, and disease do not influence the occurrence of the symptom, though, as in Case 8, the amount and duration may be increased. Acetonuria followed anæsthesia in 12 cases, in which no rise of temperature followed operation. A curious feature is that the quantity of anæsthetic and the duration of anæsthesia apparently do not affect the amount or duration of the acetonuria; in Case 21, in which the anæsthesia was maintained for 1 hour and 20 minutes, the urine had returned to the condition it was in before operation on the fifth day; whilst in Case 13, anæsthesia 15 minutes, the urine was not free from acetone until the eighth day.

In none of my cases was ether alone used, but in 148 cases reported by Becker, 48 gave a negative result; that is to say, acetone was not found in the urine tested directly; in 13 cases, in which chloroform alone was used, a negative result was obtained in 4 (Nos. 4, 5, 6, 14), but acetone was present, as was found on distillation.

These facts suggest that ether anæsthesia, though it always causes acetonuria, does so to a less extent than chloroform anæsthesia.

In 13 of my 25 cases (Nos. 1 to 6, 8, 9, 12, 13, 14, 19, 20), no acetone was found in the urine before the anæsthetic was given. This does not agree with von Jaksch's statement, but may be explained by the fact that in his cases as much as 50 litres of urine were distilled.

We may agree, then, with Becker that "acetonuria follows anæsthesia, and that if it be already present it is increased thereby."

The explanation of this phenomenon is very difficult. We know but little as to the actual production of acetone. Le Nobel¹ states that a diet rich in albumen, or the use of alcohol, leads to acetonuria, and he considers that it (acetone) is a product of albumen-destruction; to this view the occurrence of acetonuria in diabetes and fever lends support.

That albumen-destruction is the source of acetone in anæsthesia cases seems unlikely, inasmuch as the duration of the anæsthesia ought to influence the amount of the acetonuria, yet from Becker's and my own results this is not the case.

Be the source of the acetone what it may, it is probable that it is derived from ethyl-diacetic acid, or some allied substance, and it is interesting to note that in 6 of Becker's cases aceto-acetic acid was present in the urine.

Although acetone itself is non-poisonous, yet its precursors probably are, and therefore, in any subject, *i.e.* diabetics, in whom a tendency to acetone formation is marked, general anæsthesia should be induced

¹ Hammarsten, "Physiolog. Chemie."

only in cases of urgency. A number of cases are on record¹ in which coma has followed general anæsthesia, and it is obvious that if the forerunners of acetone are poisonous, then the explanation of these cases is simple; the coma is due to the poisonous principles to which the anæsthesia has given rise.

I am greatly indebted to Messrs. Armstrong and Ross, House Surgeons to the Royal Infirmary, for the trouble they have taken in supplying the urines examined.

¹ Becker, *Deutsche med. Wchnschr.*, Leipzig, Nos. 17 and 18, 1894.

NOTES ON THE OCCURRENCE OF LARGE QUANTITIES OF HÆMATOPORPHYRIN IN THE URINE OF PATIENTS TAKING SULPHONAL.¹

By ARCHIBALD E. GARROD, M.A., M.D., F.R.C.P., and
F. GOWLAND HOPKINS, M.B., B.Sc., F.I.C.

MANY cases have recently been recorded in which urine of a deep red colour has been passed, which has proved on examination to be free from unaltered blood pigment, but to contain large quantities of an iron-free derivative of hæmoglobin, namely, hæmatoporphyrin. We are not here confronted with the occurrence in the urine of an unusual or exceptional ingredient, for traces of hæmatoporphyrin are usually, if not constantly, present in normal human urine, and under a great variety of morbid conditions larger amounts are met with in specimens which nevertheless exhibit no marked peculiarity of tint. On the other hand, the quantity of the pigment present in these dark red urines is far in excess of that met with in ordinary morbid specimens.

The evidence which connects the phenomenon under consideration with the administration of sulphonal is so cogent that the connection between them is usually regarded as an established fact. The great majority of the recorded cases have occurred in insane persons, by whom this drug has been taken for a considerable period, and furthermore, the renewal of the sulphonal treatment after recovery has more than once been followed by a return of the symptoms. The allied drugs, trional and tetronal, appear, moreover, to be capable of producing a similar effect.²

Except in the slightest cases, the excretion of dark red urine is only one of a group of symptoms which appears to be connected with the administration of sulphonal. Of these vomiting, constipation (sometimes following an initial diarrhœa), and abdominal pain are the most constant. In a few cases febrile disturbance has been observed, but as a rule there is no rise of temperature. Paresis or paralysis of limbs, sometimes of an ascending type, with diminution of knee jerks, are not

¹ Paper read before the Pathological Society of London, on November 5, 1895.

² *Vide* Schultze, *Deutsche med. Wchnschr.*, Leipzig, 1894, s. 152, and Herting, *ibid.*, s. 343.

infrequently present, and ptosis and diaphragmatic paralysis have been met with.

In some instances, when the drug is stopped, the urine gradually resumes its normal colour, and recovery takes place, but in many cases the patients pass into a condition of collapse, with cyanosis, feeble and rapid pulse, and coldness of the extremities. They may sink into a condition of apathy, or may remain conscious up to the time of death, which quickly follows upon the development of these symptoms.

Very few post-mortem records are available, and the changes met with at the autopsies have not been conspicuous. Lesions of the brain have been noted, and in one case there was mitral stenosis. The liver has more than once shown advanced fatty degeneration.

In a case recorded by Percy Smith,¹ there was much post-mortem staining and injection of the vessels of the stomach and intestines, and in the ileum there were one or two patches of submucous hæmorrhage; an observation which is of interest in connection with the views of Stockvis, which will be referred to later.

On the other hand, in a case reported by Oswald,² the stomach and intestines showed nothing of special note, excepting some congestion of the first part of the duodenum, and in other parts corresponding to the coils.

In one of the cases recorded by Hammarsten³ also, although the unequal contraction of parts of the alimentary canal is carefully described, there is no mention of hæmorrhage or injection.

The kidneys have not shown any obvious naked-eye lesions, except such as could be referred to antecedent disease or senile change, but Stern and Oswald have described granular or necrotic changes in the epithelium of the glomeruli, and secreting portions of the tubules, and Kast⁴ observed hæmorrhage into the glomerular capsules in dogs poisoned with sulphonal.

That poisoning with sulphonal has some effect upon the kidneys is shown by the not infrequent appearance of albumen in the urine, when symptoms of poisoning develop, and by the presence of casts, hyaline, cellular or granular, more frequently than can be ascribed to accidentally associated renal disease.

We must not omit to mention that Franz Müller⁵ speaks highly of the therapeutic effect of alkalis, such as sodium bicarbonate, in these cases, and believes that such treatment, if adopted, as soon as the unfavourable symptoms develop, is capable of averting a fatal ending. Stockvis endorses this recommendation, and it is obvious that this method of treatment should in future be given a trial when the above-described symptoms are met with in patients taking sulphonal.

¹ *St. Thomas's Hosp. Rep.*, London, 1891, vol. xxi. p. 241.

² *Glasgow Med. Journ.*, 1895, vol. xliii. p. 4.

³ *Upsala Läkaref. Förh.*, 1890-91, vol. xxvi. p. 267.

⁴ *Arch. f. exper. Path. u. Pharmacol.*, Leipzig, 1892-93, bd. xxxi. p. 69.

⁵ *Wien. klin. Wchnschr.*, 1894, s. 252.

Assuming then, as we are apparently justified in doing, that in the great majority of instances, the administration of sulphonal is the actual cause of the condition, we are nevertheless confronted with several important difficulties.

1. The action of the drug appears to be a cumulative one, and the patients have usually taken sulphonal for a considerable period of weeks or months before any ill effects are observed. Sometimes the symptoms have only appeared after the sulphonal has been discontinued, and in one of the cases recorded by Hammarsten an interval of no less than 9 days had elapsed since the last dose was taken.

2. Under ordinary circumstances the urine of patients taking sulphonal does not contain more hæmatoporphyrin than that of other healthy or diseased individuals, as was found by one of us on the examination of specimens kindly supplied from Bethlehem Hospital;¹ and of the large number of patients who take this drug only a very few exhibit the symptoms under discussion.

3. In a *very large majority of cases* the patients who do so suffer are females. We do not know of any recorded fatal case in a male, and, indeed, have only met with the mention of two cases of a slight character in such subjects. One of these, which is quoted by Franz Müller,² was that of a man suffering from advanced tabes dorsalis, who had taken from half a gramme to a gramme of sulphonal nightly for 2 months; and the other, referred to by Percy Smith, was that of an old man, under the care of Dr. Savage, who had taken doses of 20 to 25 grs. nightly for more than a year.

In ordinary morbid cases, on the other hand, we do not find that sex has any influence upon the increase of the urinary hæmatoporphyrin.

4. Lastly, there are a few cases recorded by MacMunn,³ Ranking, and Pardington,⁴ Sobernheim,⁵ and others, in which dark red urine, rich in hæmatoporphyrin was passed by patients who had taken no sulphonal. In some of these, and notably those of Ranking and Pardington, in one of which acetanilide was given, general symptoms somewhat resembling those of the sulphonal patients were observed, but in others this was not the case. We do not propose at the present time to enter upon any discussion of the origin of urinary hæmatoporphyrin, but it is necessary to mention the views recently enunciated by Stockvis,⁶ who believes that the traces present in normal urines, and the larger amounts met with in disease, are alike derived from the blood pigment contained in the food; whereas he ascribes that present in cases of lead poisoning, and the far larger amounts met with in sulphonal urines, to hæmorrhage into the mucous membrane of

¹ *Journ. Path. and Bacteriol.*, Edin. and London, 1892, vol. i. p. 187.

² F. Müller, *loc. cit.*

³ *Journ. Physiol.*, Cambridge, 1890, vol. xi.; *Proc. Physiol. Soc.*, London, p. 13.

⁴ *Lancet*, London, 1890, vol. ii. p. 607.

⁵ *Deutsche med. Wchnschr.*, Leipzig, 1892, s. 566.

⁶ *Ztschr. f. klin. Med.*, Berlin, 1895, bd. xxviii. s. 1.

the alimentary canal; the conversion of the blood pigment into hæmatoporphyrin being in these last cases materially aided by the presence of the sulphonal which causes the hæmorrhages. Concerning these views we would here merely state that our own observations and experiments make us hesitate to accept them as offering a complete explanation of the observed phenomena, and as regards normal and ordinary morbid urines, to question strongly the correctness of Stockvis's theory. The grounds for this attitude we hope to set out fully on a future occasion.

On the other hand, the observations to be described in this paper, add in one respect to the evidence, in opposition to the view that increase of urinary hæmatoporphyrin implies excessive blood destruction, for the presence in the urines, examined by us, of large quantities of this iron-free derivative of hæmoglobin was not attended by any corresponding increased excretion of iron. Blood examinations in the sulphonal cases are few in number. Percy Smith¹ did not find in his cases any greater diminution of blood corpuscles, or of hæmoglobin, than is usually met with in cases of melancholia, from which disease the patients were suffering. In one case the number of red corpuscles per cubic mm. was no less than 4,600,000.

In a case recorded by Oswald,² three examinations of the blood, at intervals which are not stated, gave results varying between 3,520,000 and 3,150,000, a difference so small that no great significance can be attached to it. On the other hand, conspicuous anæmia is frequently mentioned in the clinical reports, and E. Schäffer³ describes a great diminution both of red corpuscles and of hæmoglobin in his case, but neither the hæmoglobinometer or hæmacytometer were employed.

Franz Müller⁴ states that in his case the percentage of hæmoglobin fell to 45, and returned to 85 per cent., after the hæmatoporphyrinuria had ceased. He, however, gives no enumeration of the corpuscles.

Since the point to be ascertained is whether the passage of large amounts of hæmatoporphyrin in the urine is *necessarily* accompanied by excessive blood destruction, it is obvious that the force of even a few observations which do not reveal any successive hæmolysis, such as those of Percy Smith and Oswald, is not impaired by the fact that in other cases there is a condition of advanced anæmia. On the other hand, it should be noted that, as Salkowski has pointed out, a given quantity of hæmatoporphyrin represents more than twenty times its own mass of hæmoglobin, and this observer calculated that in one of his cases the daily excretion of hæmatoporphyrin corresponded to about $\frac{1}{32}$ of the total hæmoglobin of the patient.

¹ P. Smith, *loc. cit.*

² Oswald, *loc. cit.*

³ *Therap. Monatsh.*, Berlin, 1893, s. 57.

⁴ F. Müller, *loc. cit.*

In all our specimens, as will be seen from the following account, very little urobilin was present, and in the third case, in which a special method was employed for its extraction, a quantity much smaller than that usually got from normal urine was obtained. Seeing that there is strong evidence that excess of urobilin in the urine accompanies excessive hæmolysis, the above fact is also significant in this connection.

The chemical and spectroscopic characters of the dark red urine of sulphonal poisoning have been carefully studied by Salkowski,¹ Hammarsten,² Stockvis,³ and MacMunn,⁴ and our object in bringing forward the following three cases which have come under our notice is to emphasise certain points to which these eminent observers have already called attention, and in certain particulars to supplement their results.

CLINICAL HISTORIES.

For the following Clinical Notes we are indebted to Drs. J. Delpratt Harris, W. M. Abbot Anderson, and M. J. Nolan, who respectively had charge of the cases, and from whom the specimens were received.

CASE 1 (from notes by J. Delpratt Harris).—Miss M. J. W., æt. 50, single; chronic epileptic since 16 years of age. Suffered much from sleeplessness, and had taken sulphonal more or less continuously since May 1889. The drug has occasionally been omitted for a night or two, or even for a week, and then, insomnia supervening, patient has always returned to the sulphonal, and has occasionally taken two doses of 20 grs. in 8 hours. It has always been found to agree well, and surpassed chloral in producing sleep; for a relatively larger amount of the latter was required to produce the effects of 20 grs. of sulphonal—her usual dose. Her sleeplessness, if neglected, resulted in attacks of epileptic mania, of which there were three in 7 years.

12th March 1895.—There is much pain in the lower part of the bowels over the region of the bladder, with tenderness over the right ovary and constant sickness. She was given a carminative mixture.

14th March.—There is some constipation. Colocynth, calomel, and belladonna were given in pill. From this date to April 10 patient was better. She was taking a tonic of gentian and nux vomica.

10th April.—Much abdominal pain again felt. It seemed to concentrate in the region of the bladder, and was increased by pressure in this region. Constant sickness and complete loss of appetite. Buchu and hyoscyamus in large doses had no effect on the pain.

13th April.—To-day it was noticed, after a fit, that the urine was of deep claret colour, but had no sediment. Temperature normal. Pulse quick and feeble. She is so weak that she cannot raise her hand from her bed. She suffers from piles, but there is little or no bleeding. Up to this date sulphonal had been taken almost every night, and on the 10th she took 40 grs. during the night. The quantity of urine averaged 2 pints daily. There was never

¹ *Ztschr. f. physiol. Chem.*, Strassburg, 1891, bd. xv. s. 286.

² *Skandin. Arch. f. Physiol.*, Leipzig, 1892, bd. iii. p. 319.

³ *Nederl. Tijdschr. v. Geneesk.*, Amsterdam, 1889, p. 413.

⁴ See Percy Smith, *loc. cit.*

any decided reaction with albumen tests. The colour was deepest about 13th April. It very gradually became less so, and by the 26th was but slightly coloured.

26th April.—Patient steadily became weaker and weaker, and very pale and anæmic, complaining of thirst, and looking like one very much exhausted from hæmorrhage. During the night she had an epileptic seizure, from which she never quite recovered, and died shortly afterwards. There was never any œdema of the extremities, or any ascites. No pulmonary symptoms until the very end, when œdema occurred. The pain and sickness were the first symptoms of any serious trouble (12th March). One month after this began the dark coloured urine was first passed, and a fortnight later death occurred. The abdominal pain was at first felt as high as the liver, later it was always over the bladder.

CASE 2 (from notes by W. M. Abbot Anderson).—Female, æt. 32; married at 20. Treated for hysteria, by Weir Mitchell method in 1892. Bad attack of erysipelas in 1887. In 1893 showed evident signs of myxœdema.

26th December 1894.—First showed signs of typhoid fever. This became of very severe type, and was followed by relapse. Ill 2 months, but well enough to go to Bournemouth at the end of February 1895. During this illness the patient slept badly, and took sulphonal in doses from 10 to 20 grs. The use of the drug continued from 26th December 1894 to early in May 1895. Towards the middle of April she returned to London from Bournemouth, and was progressing favourably. One day towards the end of April she unduly exerted herself, came home exhausted, and had to take to her bed. Shortly afterwards she passed dark red urine. From this time she continued to grow weaker and weaker, and died 11th May 1895.

CASE 3 (from notes by M. J. Nolan, County Down Asylum).—Female, æt. 33, married; millworker; admitted to Downpatrick Asylum, 12th April 1895, suffering from acute melancholia of the agitated type. The attack commenced immediately after the birth of her child, 8 months previously. This child was nursed at the breast up to a few days before admission. Physical examination gave no evidence of organic disease. She had been partially refusing food for some weeks, and had been sleepless and constipated, and was in a very low state of health. The skin of the entire body was very dark-coloured, suggestive of the bronzing of Addison's disease, and the face, in addition, was of the peculiar cachexia seen in malignant disease; frequent and careful examinations, however, failed to determine any such conditions, nor did the patient complain of any symptoms associated with the diseases named.

She was put to bed, mild aperients and abundant nourishment were given, and 20 grs. of sulphonal were administered every second night to secure sleep. Sulphonal was continued in this way for a few weeks, by which time the condition had improved. The drug was now given only at irregular intervals (in doses of 20 grs.), whenever patient was reported as awake all the previous night. There was very little change in the mental and physical state from this time to the first week in July, when a violent relapse caused sulphonal to be given twice daily (10 grs. at 12 noon, and 20 grs. at 6 p.m.) for a fortnight; but as patient did not seem to derive the benefit expected the *drug was abandoned on the 21st July, and was not administered again.*

A few days later she was quieter, and complained of weakness, which increased until the 31st July, when she was confined to bed. There was not at any time stupor, motor inco-ordination, pain, vomiting, feverishness, or gastro-intestinal irritation.

1st August.—Patient was very weak, complaining of general lumbar pain; temperature and pulse normal. For the first time the urine was noticed to be

of a brownish tinge, but as it was mixed with a small quantity of *fæces*, it was difficult to determine the true character of the pigmentation. The quantity passed was not over the average.

2nd August.—The discoloration was more pronounced, the urine being now of the shade of *old port wine*. Held up against the light it was quite tawny, showing no cloudiness or “smoky” appearance. On examination, albumen and sugar were found absent, and for the first time hæmatoporphyrin was suspected. Thus the condition began 10 clear days after sulphonal had been wholly abandoned. It continued and increased daily to the date of death, 9th of August, which was 9 days from the date of its first appearance. The clinical symptoms during these 9 days were confined to intense weakness (the pulse being very small and slow), and a change in the colour of the skin. The dark tinge of the latter became clearer, owing to the increased *anæmia*, which was indicated by a pearly white sclerotic, blanched lips and fauces. The temperature became slightly subnormal, and respiration feeble. There was no indication of nephritis, which was daily looked for. After death the skin was of a whity-brown hue. No post-mortem was allowed.

It will be observed that all three patients were females, all had taken sulphonal, and in all the cases death followed shortly after the development of the symptoms, the intervals being 13, 14, and 9 days respectively.

In Case 1 the symptoms conformed to the ordinary type. A point of special interest in this case was the long period during which sulphonal had been taken without any ill effect (no less than 4 years). The pain in the region of the bladder here complained of has been met with in other cases, and was a conspicuous feature in the cases described by Ranking and Pardington, in which sulphonal was not given. The second and third cases were peculiar in the absence of gastro-intestinal symptoms.

In Case 3, as in one of Hammarsten's cases, above referred to, an interval of no less than 10 days elapsed between the administration of the last dose of sulphonal and the onset of the symptoms. In connection with the pigmentation of the skin in Case 3, which was present before any sulphonal was taken, it is interesting to note that caseation of the suprarenals and bronzing were present in a sulphonal case, referred to by Oswald as having occurred in the Edinburgh Royal Infirmary. We much regret that we have no post-mortem observations to add to the few already published.

GENERAL CHARACTER OF THE URINE.

In all 3 cases the specimens came into our hands for examination only a few days before the death of the patient. Any attempt to make a series of observations, either on the pigmentation or general condition of the urine, was therefore prevented. The specimens examined were passed when the condition of dark urine was at its height. Their colour was that of port wine. All the specimens were acid, and of low specific gravity (1010–1013). The percentage of urea

was about the same in each (2 per cent). In none was more than a minute trace of albumen present; one specimen from Case 1 was in fact entirely free; Case 3 showed more than the others, but the quantity was extremely small. In spite of this, the deposits obtained by use of the centrifuge contained abundant tube casts. In the absence of any renal symptoms, and in the practical absence of albuminuria, this fact seemed somewhat surprising; but the literature of the subject as stated above shows that the presence of casts is common in these cases. In ours they were very numerous, indeed. In addition to the ordinary hyaline and epithelial varieties, there were many well-formed, finely granular casts, of purplish colour, which appeared to be almost wholly made up of pigment. The cells composing the epithelial casts were deeply pigmented, and the deposit comprised numerous isolated epithelial cells, and many large granular leucocytes, all of which contained much pigment. This description is true of all three cases.

ABSENCE OF IRON FROM THE URINE.

It seemed a point of some interest to determine whether the great increase in the excretion of the iron-free derivative of hæmoglobin was accompanied by any increased excretion of iron, present in some other combination. In Case 1, 8 oz. of urine were evaporated to dryness, great care being taken to prevent contamination with extraneous iron. The residue was burnt in a muffle-furnace, the ash dissolved in hydrogen chloride, and, after the addition of a little hydric nitrate, the solution was evaporated to dryness. Taken up again in a little dilute hydrogen-chloride and filtered, the solution was made just alkaline with ammonia, and boiled. The ammonia precipitate was filtered off, dissolved in 2 c.c. of dilute hydrogen chloride, the solution divided into two parts, and tested (*a*) with sulphocyanide, and (*b*) with ferrocyanide of potassium. *Neither reagent gave the least trace of colour.* Eight oz. is perhaps a somewhat small quantity to use for this purpose, but control specimens of normal urine gave in several cases distinct iron reactions from the same quantity, so there can be no doubt that any increase in the urinary iron was absent from Case 1. The other cases (in which, however, still smaller quantities had to be used) also gave negative results.

SPECTROSCOPIC EXAMINATION OF THE URINE.

CASE 1—*Specimen A.*—The urine had a deep port-wine colour. In a layer of 1.5 cm. a band was seen in the red from λ 624 to λ 612. This is the first band of alkaline hæmatoporphyrin—from the D line onwards the spectrum was completely obscured.

Diluted with an equal quantity of water in a depth of 1.5 cm. the following bands were seen :—

- | | |
|-----------------------|-----------------|
| 1. λ 624–612 | } rather faint. |
| 2. λ 589–573 | |
| 3. λ 546–532. | |

From D onwards towards the blue the spectrum was much obscured. Absolute darkness extended from a band λ 515.

On the addition of a few drops of hydrogen chloride the bands read :—

- | | |
|-----------------------|-----------|
| 1. λ 597–589. | } shading |
| 2. λ 576–570. | |
| 3. λ 558–540. | |

These are the bands of acid hæmatoporphyrin. There was darkness from λ 529.

On shaking with acetic ether a pink ethereal extract was obtained, which showed the following bands :—

- Shading to λ 642.
1. λ 628–620.
 2. λ 601–597.
 3. λ 586–569.
 4. λ 540–525.
 5. λ 511–482.

This is a slightly modified neutral hæmatoporphyrin spectrum.

The subjacent liquid, after treatment with the acetic ether, was of deep brown colour, showing complete absorption of the spectrum from λ 549.

On the addition of a drop of hydrogen chloride the pigment left the acetic ether for water, forming a deep pink solution, which showed the acid hæmatoporphyrin bands with great intensity. No urobilin band. Readings—

- λ 597– λ 589.
 λ 576– λ 560–543.

A more concentrated specimen, similarly treated, showed a faint urobilin band.

The soda method and the ammonium chloride method were quite unable to cope with the amount of hæmatoporphyrin here present, the precipitates being able to carry down only a small portion.

The extract from the soda precipitate was peculiar in that the hæmatoporphyrin could not be, by any means, persuaded to go into chloroform.

Specimen B.—This was much darker than the earlier specimen just described. The colour was almost black. Shaken with acetic ether, it yielded an extract less distinctly pink than that obtained by the same method from Specimen A (*v. supra*). After extraction with

the acetic ether the urine retained a deep brown colour, and absorbed the spectrum quite up to the red.

About 4 oz. were treated by Salkowski's barium method. The precipitate had a deep mauve colour, the filtrate was yellow. The precipitate was washed and treated with alcohol acidified with hydrogen sulphate.

The extract had a very deep colour. From the acid liquid, after the addition of water, chloroform took a very deep red colour. The supernatant liquid was poured off and water substituted. On shaking, a mahogany-brown pigment left the chloroform, and also a little hæmatoporphyrin. After repeated washing, the chloroform was pink, and showed the five-banded spectrum of neutral hæmatoporphyrin with great intensity.

A specimen of the *original urine* was repeatedly extracted with acetic ether.

The first and second ethereal extracts were red-pink, and showed the following bands:—

1. λ 628–624, fainter.
2. λ 586–570
3. λ 549–526 } very dark.
4. λ 508–486, faint.

The appearances suggested that much two-banded (oxy-hæmoglobin-like) hæmatoporphyrin was mixed with a small quantity of the ordinary kind.

The third ethereal extract was reddish-brown, and showed the two dark bands very clearly. No band was seen in red, and that in blue was very faint.

CASE 2.—The urine had a dark port-wine colour. Filtered, and examined direct in a layer of 2.25 cm., the specimen allowed only a little red to penetrate. With a wider slit a band was seen—

λ 624–612, darkness from D.

On dilution—

1. λ 624– λ 612, faint.
 2. λ 586– λ 573.
 3. λ 549– λ 529.
- General darkness from λ 515.

On adding hydrogen chloride the acid hæmatoporphyrin bands appeared—

1. λ 597–589.
2. λ 560–543.

On shaking with amylic alcohol, a very intense spectrum of alkaline hæmatoporphyrin was obtained—

1. λ 626–618.
2. λ 597–586–568.
3. λ 549–532.
4. λ 515– ?

The extract was red, the subjacent fluid dark brown.

Salkowski's barium method gave a red precipitate, the filtrate being pale yellow.

The precipitate was washed and treated with alcohol, acidified with hydric chloride.

The acid extract had a deep red colour (not pink), and showed the acid bands well, and with ammonia the alkaline bands strongly, but the pigment could not be got to go into chloroform.

The Salkowski extract was neutralised with ammonia, and evaporated nearly to dryness. On adding a drop of hydrogen chloride and shaking with acetic ether, the ether became pink, and showed a three-banded spectrum, as follows:—

1. λ 570–536.
2. λ 532–517.
3. λ 506–481.

A specimen of the same urine shaken with acetic ether gave a reddish-pink extract, which showed the five-banded neutral spectrum:—

1. λ 628–620.
2. λ 601–592.
3. λ 586–567.
4. λ 549–526.
5. λ 513–484.

CASE 3.—The colour of the specimen resembled that of dark port-wine.

A portion was repeatedly extracted with acetic ether. The extracts were red, and showed the bands of alkaline hæmatoporphyrin with decreasing intensity in each successive extract.

After three extractions the urine no longer showed these bands, but still had a dark red colour. This specimen, from which almost all the hæmatoporphyrin had been extracted, when treated with barium-chloride and hydrate (Salkowski's process) gave a purple precipitate, which, when treated with alcohol, acidified with sulphuric acid, gave a reddish-brown extract, which showed the bands of acid hæmatoporphyrin very faintly, and much general absorption from the green onwards. On the addition of ammonia the liquid became brown, and no distinct bands were seen.

The original urine, diluted with water, showed a band in red (λ 6200–6100), a shading from λ 6010–5825, and complete absorption beyond. In a thinner layer, the following spectrum was seen:—

1. λ 6200–6100, faint.
2. λ 5860–5570, faint.
3. λ 5430–5320, very dark.
4. λ 5080–4690, very dark.

These are the bands of alkaline hæmatoporphyrin, but the great relative intensity of the third and fourth bands, and a shading

connecting them, showed the presence of a second pigment. On the addition of sulphuric acid, the bands of acid hæmatoporphyrin appeared, and also a very broad band with ill-defined edges from about λ 5290–4690.

To another portion more than its own bulk of rectified spirit was added, which caused turbidity, and, on filtering, a brown precipitate was collected. This yielded hæmatoporphyrin to alcohol, acidified with acetic acid, showing that in this, as in Stockvis's case, some of the hæmatoporphyrin was insoluble in neutral alcohol.

The residue upon the filter was readily dissolved by a dilute solution of caustic soda in water, yielding a brown solution which showed the following bands:—

1. λ 6200–6100, faint.
2. λ 5760–5570, faint.
3. λ 5460–5370, dark.
4. λ 5170–4910, dark.
5. λ 4720–4570, faint.

Here the third and fourth bands were far darker than the others, which agree with those of alkaline hæmatoporphyrin, the fifth band in the extreme violet being one which is only seen when an excess of alkali is present. This band disappeared on the addition of acetic acid.

The dark third and fourth bands were those which were so conspicuous in the original urine, and the spectrum was obviously either a modification of that of alkaline hæmatoporphyrin, or that of a mixture of two pigments, one of which was ordinary hæmatoporphyrin. That the latter was not the case was shown by the fact that on the addition of sulphuric acid the bands of acid hæmatoporphyrin did not appear, but only an ill-defined absorption band from λ 5290– λ 4770.

Another portion of the original urine treated by Salkowski's method yielded a brown precipitate, from which a deep red alcoholic extract was obtained, which showed the bands of acid hæmatoporphyrin with great intensity, but there was also much general absorption from the green onwards. On the addition of ammonia, the liquid became much paler, brown in colour, and showed the bands of alkaline hæmatoporphyrin. Only a trace of urobilin was present in this urine.

It should be mentioned that in this case the examination of the urine was not made until some weeks after it had been passed, and there was reason to believe that when fresh it contained little, if any, ordinary hæmatoporphyrin. Such development of the pigment in the urine, on standing, has been noticed by several observers.

In all the above specimens there was evidence of the presence of a reddish-brown pigment, producing a great general absorption of the more refrangible portion of the spectrum, but showing no bands.

Owing to this, the urine always remained deeply coloured after all or nearly all the hæmatoporphyrin had been extracted.

In reviewing the results of the above examinations, one point to which we desire to call attention is that the methods which we have found most serviceable for the detection of hæmatoporphyrin in ordinary morbid urines (viz. that by the addition of caustic soda, and extraction of the pigment from the washed phosphate precipitate, and that by saturation of the urine with ammonium chloride, and treatment of the urate precipitate with a mineral acid) are useless when we are dealing with such large quantities of the pigment as were present in the urines above described. Both the methods referred to—the former especially—are extremely delicate, and serve for the detection of the traces present in normal urines, but they are not true precipitation methods, the pigment being merely carried down upon the precipitates of phosphates and urates respectively, to which, however, it clings with sufficient tenacity to allow of the washing of the sediments.

Either of these methods, when applied to the sulphonal urines, leads to the separation of only a very small part of the contained hæmatoporphyrin, which is here present in quantities which completely overtax the carrying power of the precipitates.

Salkowski's method of precipitation with barium chloride and hydrate removes all the abnormal pigments, leaving the filtrate of a pale yellow colour, but, as we have seen, much of the pigment so carried down is, in most instances, not hæmatoporphyrin.

We have found the method above described, of repeated extraction with acetic ether, very useful for the separation from each other of the abnormal pigments, and the earlier extracts so obtained contain much less of the pigments other than hæmatoporphyrin than do similar extracts obtained by shaking the urine with amylic alcohol.

Another point upon which, in our opinion, sufficient stress has not hitherto been laid, is this, viz. that the deep colour of these sulphonalurines is *only in part* due to the hæmatoporphyrin which they contain.

A specimen of urine may show, when examined with the spectroscope in a sufficiently deep layer, the entire spectrum of the so-called alkaline hæmatoporphyrin so distinctly that the bands can be accurately measured, and yet have merely a rich orange colour; nor have we been able, by adding to normal urine isolated urinary hæmatoporphyrin in such quantities that the absorption bands were seen with great intensity, to reproduce at all the colour of the sulphonal specimens.

On the other hand, in the sulphonal urines, the bands of hæmatoporphyrin—although, as far as the general absorption allowed them to be seen, they were quite distinct—did not in any way

correspond in definition to what might have been expected, if the tint of the liquid had been largely due to that pigment. In some specimens the quantity present was much larger than in others, but those which contained most hæmatoporphyrin were not the darkest in colour.

In some instances there was reason to believe that much of the additional abnormal colouring matter was in the form of derivatives of hæmatoporphyrin, but even this cannot be asserted of all the cases.

In Case 1 there was present, in addition to much ordinary hæmatoporphyrin, a large quantity of a pigment, undoubtedly allied to it, which showed two absorption bands resembling those of hæmoglobin, and in this the specimens agreed with that examined and described by Stockvis, who separated the two-banded pigment from the ordinary hæmatoporphyrin by dialysis. The two-banded pigment under discussion resembled that found by one of us in urate sediments from urines fairly rich in hæmatoporphyrin, and a similar spectrum is yielded by the zinc compound of that pigment.

In all three cases the colour of the urine appeared to be in great part due to a reddish-brown pigment, which showed no bands, but largely absorbed the violet end of the spectrum. It seems highly probable that this was identical with the reddish-brown pigment found by Hammarsten in three out of the four specimens which he examined, and to which he also attributed an important share in their coloration. This is a point of some importance, since there is a tendency to ascribe to any band-yielding pigment or pigments which they may contain an undue share in the coloration of specimens of urine. We see this well exemplified in the ascription of the colour of normal urine to urobilin, a pigment which, in solutions so dilute as to show a band such as yielded by normal urines, when, indeed, they yield a band at all, has hardly any appreciable tint. We are also prepared to maintain that the dark colour of the urine in cases of pernicious anæmia is only in small part due to the excess of urobilin which it is wont to contain.

In Case 3, as in Stockvis's case, a considerable quantity of the abnormal pigment was precipitated by the addition of an excess of alcohol, and the precipitate so formed consisted in part of hæmatoporphyrin which was taken up by alcohol acidified with acetic acid, and in part of a brown pigment, readily soluble in alkalies, and which showed a spectrum somewhat resembling that of alkaline hæmatoporphyrin, but which, on the addition of a mineral acid, yielded only a broad and very ill-defined absorption of the blue and green.

It is found that samples of hæmatoporphyrin, derived from different urines are apt to exhibit curious differences both from each other and from specimens prepared from hæmoglobin. Such slight differences as relate merely to displacements of the absorption bands can be explained as the results of differences of solvent, of

the degrees of acidity or alkalinity of the solutions, and such-like causes, but, as we have seen, there are also observed remarkable differences of solubility, examples of which have been quoted above, and are perhaps more conspicuous in dealing with the pigments from these dark red urines than with those from ordinary morbid specimens.

We may quote the precipitation by alcohol, in which liquid hæmatoporphyrin is usually freely soluble, and the fact that the hæmatoporphyrin present in Cases 1 and 2, unlike any other specimens that we have ever met with, refused entirely to go into chloroform out of an aqueous-alcoholic solution acidified with acetic acid.

Such differences have been observed by all who have made a special study of such urines, and we have yet to learn how far they are due to actual differences in the pigments themselves, and how far to the disturbing influence of impurities present in the solutions dealt with.

A STUDY OF THE HUMAN PLACENTA, PHYSIOLOGICAL AND PATHOLOGICAL.

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(PLATES XIX. TO XXII.)

PART I.—DEVELOPMENT AND NORMAL STRUCTURE.

THE object of this paper is to give some account of the minute structure of the human placenta, with especial reference to its development. For the sake of brevity, a description of the macroscopic characters of the placenta is omitted, and some of the better known points of histology are only briefly alluded to.

For reasons which will afterwards appear, the normal structure of the placenta must be studied in specimens obtained not later than the mid-term of gestation. This paper is therefore not concerned with the placenta at the full term. Frequent references are made to the very voluminous literature of the subject, and a list of all such references will be found at the end of the paper.

The placenta consists of two distinct series of structures; one developed from the ovum, *the foetal placenta*; the other developed from the uterus, the *maternal placenta*. The two parts, of which the placenta is composed, are best studied separately.

A. *The Foetal Placenta.*

The foetal placenta is an elaboration of the outer foetal envelope or chorion; it is therefore necessary, in the first place, to trace briefly the development of this membrane.

Very few facts are known concerning the development of the human ovum, during the first 14 days of its existence. Reichert⁽³⁶⁾¹ and His⁽¹⁸⁾ have described human ova of about 12–13 days; in them the decidua reflexa was fully formed, and chorionic villi

¹ The numbers in brackets refer to the Bibliography at the end of the paper.

were present over the greater part of the surface of the ovum. By what precise steps this stage is reached, in man, is not known. We can only assume that it is by a process which does not materially differ from that of mammals lower in the scale than man. We therefore conclude that two structures are chiefly concerned in the formation of the human chorion—(1) the *zona pellucida* or primitive envelope of the ovum, and (2) the *subzonal membrane*, or *false amnion*, which is applied to the inner surface of the *zona pellucida*, forming a complete sphere within it. The latter envelope is formed, together with the true amnion, from the extra-embryonic portion of the somatopleure. It is composed of an outer epiblastic and an inner mesoblastic layer. Kölliker (²¹).

There has been great conflict of opinion upon the relations of the allantois to the chorion. The old view was that the allantois grew out from the posterior end of the primitive alimentary canal to the wall of the ovum, in the form of a hollow process, surrounded by mesoblastic tissue; that a direct connection was thus established between the embryo and the envelope, and that along this bridge vessels developed from the terminal bifurcations of the aorta, which vascularised the chorion. This view has recently been opposed by His, and in point of fact there are no direct observations to support it. His (¹³) points out, among other objections to the theory, that allantoic or umbilical vessels have been found reaching the chorion by a mesoblastic stalk at a period when the allantois itself is only beginning to appear. Without entering into the discussion further, the view of His may be briefly stated, and adopted as most in accord with the facts as they stand at present.

The old view assumed that, after the closure of the amnion, a connection was established between the embryo and the wall of the ovum by the outgrowth of the allantois. His believes that by the closure of the amnion the fœtus is not entirely separated from its envelopes, but remains in connection with them through a bridge of mesoblastic tissue, which is continuous with its posterior extremity. This bridge he terms the *ventral stalk*. Along it the umbilical vessels grow to reach the chorion. At a later period the allantois grows out towards the chorion, in contact with the ventral stalk; but it never quite reaches the wall of the ovum. It participates with the ventral stalk and the umbilical vessels in the formation of the umbilical cord.

The chorion is thus derived from the embryonic epiblast and mesoblast layers; the epiblast forms the epithelial layer covering the entire membrane and its villi; the mesoblast forms the connective tissue stroma supporting the vessels. The *zona pellucida* probably disappears early.

In the earliest specimens of the human ovum which have been carefully examined (about the end of the second week), villi were found over nearly the entire surface of the chorion; they, therefore, probably appear at a very early period. They are formed, in the first place,

as simple ectodermal buds, which grow by a process of repeated branching, and are arborescent when fully formed. In Reichert's ovum, the villi were not found covering the entire surface (³⁶). His found them fairly evenly distributed (¹³). There is, at this period, no trace of the differentiation of a special area to form the placenta. Many of the villi are vascularised in all parts of the ovum.

This represents all that is at present known of the development of the chorionic membrane in man. It is closely related to the development of one of the maternal envelopes of the ovum, the decidua reflexa. In a human ovum of the end of the second week, the reflexa has closed completely over the ovum, and the chorionic villi are embedded in it, but so lightly that the two are readily separable. Observations are greatly needed as to the precise relations of the villi to the decidual tissues at this period; beyond the fact that there is a delicate union between them we know nothing. It is, however, a fact of great physiological importance, that, by the end of the second week, the foetal and maternal vessels are brought into close relations by the embedding of the villi in maternal tissues, thus forming what is practically a simple type of placenta in which the wall of the entire ovum is concerned.

The conclusion seems inevitable that the chief function of the decidua reflexa is associated with the nutrition of the ovum. It is a highly vascular membrane, richly supplied with glands, and the foetal vessels enclosed in their delicate villi become buried in its substance. An interchange by osmosis between the maternal and foetal blood must inevitably result. No doubt a secondary function of the reflexa is to support the rapidly growing ovum.

At the end of the second week, therefore, we have in the human ovum a simple form of diffused placenta, corresponding to that which is met with in the sow, the mare, the cetacea, etc. In the mammalia generally the umbilical vesicle plays a comparatively unimportant part in the nutrition of the embryo; and early provision is therefore made for the direct transmission of nutrient materials from the blood of the mother to the embryo. This provision, as we have seen, is found in the embedding of the foetal villi in maternal tissues. In this manner the foetus is nourished, while time is gained for the development of the more highly specialised discoidal placenta. Text-books usually state that the placenta does not appear, in the case of human ovum, till the end of the second month. I am disposed to believe, from specimens which I have examined from the middle of the second month, that the placenta is at that time already in an advanced stage of development. The point, however, is not of great importance, for the development of the discoidal placenta in man, simply consists in the specialisation of a part of the chorion to perform the work which, in earlier stages, is done by the whole of it. At the placental site the villi increase very much in size and number, and in the complexity of their branchings; at the same time important changes, to be afterwards described, occur in the

underlying decidua. As the discoidal placenta develops, the villi covering the general chorionic surface atrophy and become devascularised, and by the end of the second month this process is already complete. A diminution of the total area of the placenta is thus compensated for by the specialisation of a part of it.

It is, therefore, possible to account in a fairly satisfactory manner for the nutrition of the ovum from the end of the second week onwards. One point in this connection must be left over for future consideration, namely, the relative part which is played in the nutrition of the ovum by the glands and by the blood vessels of the maternal tissues. But there remains the important question: How is the ovum nourished during the first two weeks of its existence before the formation of the allantoic circulation? During these two weeks the ovum increases to twenty-five times its original diameter. The suggestion that, as in the case of the Aves, there is stored up in the minute human ovum an amount of nutrient material, sufficient to accomplish these results, seems incredible. The true solution is probably to be found upon quite other lines. A Dutch observer, Hubrecht, has in a recent research ⁽¹⁸⁾ upon the placentation of the hedgehog, thrown a good deal of light upon the question of the early nutrition of the ovum. In the earliest stages of the development of the hedgehog, Hubrecht describes a layer of very vascular spongy tissue surrounding the ovum, which he terms the trophosphere. This trophosphere is formed in part from the ectoderm of the blastocyst, and in part from the adjacent cellular tissue of the decidua. It contains large sinuses full of maternal blood; the outer layer of the foetal ectoderm, as well as the decidual portion, is thus richly supplied with maternal blood. When the vitelline circulation is established, primitive mesoblastic villi are formed, containing branches of the vitelline vessels, which push their way into the tissues of the trophosphere, and thus bring the foetal and maternal blood vessels into close contact. There is in fact, in the hedgehog, a vitelline placenta formed, consisting of a foetal portion with villi, and a maternal portion with large blood sinuses. Later on, as the allantoic circulation develops, the vitelline placenta disappears. It may be, of course, that the vitelline placenta of the hedgehog has no homologue in the human ovum, but Hubrecht's observations are, at least, highly suggestive.

To return now to the formation of the discoidal placenta. During the second month its formation is begun; after the mid-term it undergoes numerous retrograde changes, which will be described in a subsequent paper. The normal structure of the placenta must therefore be studied in specimens taken from the third and fourth months.

At the third or fourth month the foetal placenta consists of a dense forest of tree-like structures, having many complicated branches. Their base of attachment is the wall of the ovum. The villi are the free terminal and lateral buds, and the divisions upon which they grow represent the twigs and branches. In their growth adjacent branches often become

interlaced. The villi of the human placenta are somewhat club-shaped structures, with a rounded apex and a constricted base. Most of them are free; many, however, reach the decidua serotina and become embedded in it, forming a direct union of the foetal and maternal structures of the placenta. No one has attempted to compute the number of villi present in a fully-developed placenta, but it must be very large.

The portion of the chorion concerned in the formation of the placenta is commonly called the *chorion frondosum*; it consists of two parts, a membrane or layer, underlying the amnion, and the branching structures which spring from it. Each part of the chorion frondosum consists of three sets of structures—

1. An outer covering of epithelium.
2. A delicate connective tissue stroma, supporting
3. The blood vessels.

Each of these structures must be considered in some detail.

1. *The chorionic epithelium*.—Great confusion existed for a long time regarding the nature of the epithelial covering of the villi, and the chorionic membrane generally. Although considerable progress has been made in clearing up disputed points, there are still some matters of the first importance which remain unsettled.

In a well-preserved specimen of a young placenta, it is easy to demonstrate the presence of a double layer of cells covering the villi and all other parts of the chorion (Plate XXI. Fig. 7). This double layer has been observed as early as the second week (¹⁸, ³⁸). After the mid-term of gestation marked changes occur, the deep layer disappearing more or less completely. The appearances, therefore, vary greatly at different periods, and to this circumstance a great part of the confusion which existed on the subject was due. Observers advanced different views,¹ for the reason that they based their observations upon specimens taken from different periods of development.

The superficial layer consists of a thin stratum of granular, multi-nucleated protoplasm, in which no cell outlines can be distinguished (Plate XXI. Fig. 7). The whole layer stains deeply; its nuclei are small, round, deeply-staining bodies, placed at irregular intervals; its protoplasm is in places prolonged between the underlying cells of the deep layer. This layer is now often called the syncytium, a term introduced by writers upon the comparative anatomy of the placenta.

In the deep layer the cells are large and well-defined, with oval nuclei standing with their long axes at right angles to the surface. The intranuclear network stains very distinctly. The perinuclear proto-

¹ In a recent work (³⁹), published since this paper was written, Dr. Clarence Webster has called this layer the trophoblast, a term which he has adopted from Hubrecht's work on the placentation of the hedgehog (¹⁶). Dr. Webster's description of the trophoblast in extra-uterine gestation, and that given here of the chorionic epithelium of the uterine placenta, are in all important respects identical.

plasm is often retracted from the nucleus. There is no basement membrane present (Plate XXI. Fig. 7).

There is now a complete agreement upon the existence of these two layers of cells, and their general characters. There has been, however, and still remains, the greatest difference of opinion upon their morphology. The following examples, which comprise the most important views which have been advanced, will serve to show the extent to which this divergence of opinion has been carried:—

1. Both layers of foetal origin; the superficial epiblastic, the deep mesoblastic. Langhans (²⁷).

2. The superficial layer of maternal origin, from the endothelium of the decidual vessels; the deep layer from the foetal epiblast. Winkler (⁴⁵).

3. Both layers of maternal origin; the superficial from maternal endothelium, the deep from maternal connective tissue. Tafani (⁴⁷).

4. Both layers of foetal origin, from the epiblast. Kastchenko (²⁸). Minot (³²).

5. The superficial layer of maternal origin, being a prolongation of the syncytium deciduae; the deep layer from the foetal epiblast. Ercolani (⁶), Turner (³⁹), Merttens (³¹).

This list is by no means exhaustive; some observers have found only a single layer of cells; others have found three; and all have constructed theories to account for what they found.

It is here unnecessary to discuss these theories in detail, and, inasmuch as it is impossible in the present state of our knowledge to come to a final conclusion, such a discussion would be of little service. My own observations do not bear upon the point, but, from a careful consideration of the work of others, I believe that there are no well-supported facts which point to the origin, in the human placenta, of either of these layers of cells from maternal structures. The superficial layer I regard as epiblastic; the origin of the deep layer is perhaps more doubtful, but there are, undoubtedly, certain facts which strongly suggest the mesoblastic origin of these cells. These facts were originally pointed out by Langhans (²⁷), and it is his view which seems to me to be most in accord with the facts as they at present stand.

In animals considerably more progress has been made in determining the origin of the cellular covering of the chorion. Several observers have proved that in the dog, cat, mouse, etc., the superficial layer is in reality a maternal tissue (⁴⁴). They trace the formation in the decidua of masses of deeply-staining multinucleated protoplasm, which they have named the syncytium. These plasmodia occur as isolated masses, or as large areas or tracts, and appear to be developed from decidual cells, or from surface or glandular epithelium. Their characters are identical with those of the superficial layer of the chorionic epithelium, and the syncytium may be traced passing in direct continuity from the decidua

to the surface of the villi. These observers, therefore, term the superficial layer the syncytium, and regard it as a maternal structure.

The only observers who have attempted to prove a similar origin of the superficial layer in the human placenta are Kossmann (²⁴) and Merttens (³¹). Merttens' observations must be regarded, however, as extremely unsatisfactory. They were made upon a 16 days' ovum, obtained by curetting from a patient suspected of malignant disease of the uterus, who had suffered for some time from a profuse discharge. Observations made upon such unsuitable material as this can lead to nothing but confusion, and some caution must always be exercised in applying to the case of man the results of comparative anatomy. The question can only be satisfactorily settled by observations upon the human ovum in the earliest stages of development, and in a healthy condition.

Returning now to the structure of the chorionic epithelium, we find that during the early months the superficial layer shows great activity. Marked proliferation occurs in definite localised areas; this proliferation results in the formation of nodes or thickenings of various sizes, or of club-shaped or broad-based processes upon the epithelium. These nodes consist, like the superficial layer itself, of masses of nucleated protoplasm, in which cell outlines cannot be made out (Plate XIX. Fig. 3, and Plate XXI. Fig. 9). The deep layer appears to take no part in the formation of these buds, which are found most numerous upon the villi, but are present, in the earliest stages, in all parts of the chorion. In most of the buds the nuclei are crowded together in the centre, with a clear area of protoplasm surrounding them. Sections of processes are frequently seen as free islets of nucleated protoplasm in the intervillous spaces. Neighbouring buds often unite, forming a bridge across the intervillous space, and this process of fusion may go on to the formation of large areas, in which several villi lie embedded.

These appearances were at one time entirely mistaken. The free islets were regarded as sections of processes of decidual tissue growing into the placenta, the areas of fusion as large decidual processes in which villi had become embedded. And upon this interpretation was based the theory that the placenta developed by mutual ingrowth of foetal and maternal structures.

In point of fact, these proliferating areas represent the various stages and modifications of the process of budding, by which new villi are formed from the parent stems. The steps by which an epithelial bud becomes a vascularised villus can be readily traced in a young ovum, and are represented in Plate XIX. Fig. 3, and Plate XXI. Fig. 9, which are taken from an ovum of the sixth week. Small cavities first appear in the plasmodium, which enlarge, and fuse with neighbouring ones to form considerable rounded spaces. In the larger spaces blood may often be found (Plate XIX. Fig. 3 D), showing that they are in fact elementary blood channels. At first there is no definite

wall to the blood channel; it is bounded only by protoplasm. Next, a more or less complete ring of nuclei may be traced around it (Plate XXI. Fig. 9), and finally, a complete ring of rather large-celled endothelium is formed (Plate XIX. Fig. 3 D). In a large bud, two or three sections of blood channels may be found lying in the plasmodium without any trace of supporting connective tissue. Later, a delicate reticulated tissue appears around them, the nuclei of the plasmodium become arranged peripherally, forming the superficial layer of the chorionic epithelium, and the structure of the villus is complete. The connective tissue stroma appears to grow out into the bud from the parent villus (Plate XIX. Fig. 3 B). It seems probable that the vacuoles in the bud open into the meshes of the stroma of the parent villus; these meshes, as will be shown later on, contain abundance of free blood, which makes its way into the channels formed in the bud. These channels are not provided with definite walls until a later period, when the connective tissue grows into the bud.

All the epithelial buds which are formed do not become villi; large numbers of them undergo retrograde changes, which become of great importance in the structure of the full-time placenta, but need not be referred to here. At this period, too, the epithelium is much altered, the superficial layer has lost many of its nuclei, and is everywhere much thinned, and in places absent; the deep layer is represented only by clumps of atrophied nuclei, scattered here and there beneath the altered superficial layer.

The part played by the superficial layer of the chorionic epithelium in the formation of new villi is one of the strongest arguments in favour of the view that it is a structure of foetal, not of maternal origin. It is impossible to conceive of a tissue of maternal origin taking the initial and fundamental steps in the development of new villi from parent stems. Much greater importance must in any case be allowed to facts such as these, than to analogies drawn from comparative anatomy.

2. *The connective tissue stroma* forms the substance of the chorionic tree and supports the blood vessels. In a placenta of the second or third month it forms a delicate reticulated tissue, composed of nucleated masses of protoplasm, with numerous anastomosing processes (Plate XIX. Fig. 3, Plate XXI. Figs. 7 and 8). The nuclei are large, and the amount of perinuclear protoplasm is relatively small; the intranuclear network is very distinct. Leucocytes are present in large numbers at this period. The interstices of the reticulum appear to form a system of channels, probably of the nature of lymphatics, communicating with the capillaries; they can be injected artificially from the chorionic vessels, and red blood cells are sometimes detected in them. In the larger divisions of the chorionic tree the tissue becomes more compact; numerous spindle cells and connective tissue fibrils appear, especially in the neighbourhood of the vessels. In a placenta

at term, the whole stroma has become changed into the type of ordinary connective tissue, and the leucocytes have mostly disappeared.

3. *The chorionic vessels.*—The distribution of the branches of the umbilical vessels upon the surface of the placenta is well known and need not be recapitulated. The arterial branches dip suddenly from the foetal surface into the placental tissue; none of them reach to within half an inch of the margin. Upon the placental surface the arteries give off a number of minute twigs, which ramify in the sub-amniotic layer of the chorion, and with their corresponding veins form a complete circulation, whose function is the nutrition of the chorionic membrane, Holl (¹⁴). Beneath the surface they penetrate the placenta in a series of four or five terrace-like steps; they then branch repeatedly, and the branches do not anastomose. These branches pass into the ramifications of the chorionic tree, and terminate in the vascular loops of the villi. In the larger divisions of the chorionic tree the vessels lie in the axis; in the villi they are placed for the most part immediately beneath the epithelium, where they run a tortuous course and anastomose freely. It seems doubtful whether the loops in the villi are true capillaries, that is, channels of transition between arteries and veins; for in many instances the efferent vessel of one villus appears to become the afferent vessel of the next.

All these points may be made out in a normally shed placenta by injections of carmine-gelatine. Almost better results may be obtained by a simple device, which furnishes a natural injection of the vessels. The cord is tied at the moment of birth of the child, before respiration has aspirated the placenta. When the afterbirth has come away, the blood is squeezed back from the cord towards the placenta, and the cord is tied again at its point of insertion. The whole placenta is immersed in Müller's fluid for 24 hours; portions may then be cut out with very little escape of blood, and hardened by the usual method. Illustrations are shown of sections prepared by both of these methods (Plate XIX. Fig. 4, Plate XXI. Fig. 10).

A section of an injected placenta thus prepared shows, in the larger branches of the chorion, the axial vessels of large size, and numbers of small vessels lying peripherally, which form a superficial network; branches may be traced running off from the axial vessel to the surface. A delicate thread of injection may often be traced in the elementary vessels of the epithelial buds. In the villi of a carmine-gelatine injected placenta the meshes of the stroma are extensively injected, and this without any evidence of rupture of vessels having occurred (Plate XIX. Fig. 4). And in the naturally injected placenta red blood cells are frequently found lying in the meshes of the stroma of the villi. These appearances indicate the existence of a system of lymphatic channels in the stroma, communicating freely with the vessels of the villi. Small capillary twigs are often found, which appear to empty themselves directly into the stroma (Plate XIX. Fig. 4). The villi are therefore saturated with

blood like a soaked sponge. No escape of the injection into the intervillous spaces has ever occurred in my specimens. I have entirely failed to find any definite communication between the vessels and these lymphatics, although I am inclined to think that such must exist.

The minute structure of the vessels deserves some notice. In a young placenta from the third month the capillaries of the larger villi possess a layer of large-celled endothelium, almost cubical in type. In the small villi the tortuous capillaries always possess exceedingly delicate walls, consisting of a single layer of endothelium, supported directly by the connective tissue (Plate XIX. Fig. 4, and Plate XXI. Fig. 12). When they are full of blood, the capillaries appear to occupy nearly the whole of the villus. As a rule they run along the periphery, the only structure intervening between them and the intervillous spaces being the chorionic epithelium (Plate XIX. Fig. 4). In a full-time placenta many of the capillaries are actually exposed by the atrophy and disappearance of this covering. In the larger divisions of the chorion the vessels show some condensation of tissue around them, representing an attempt at the foundation of a tunica adventitia. The largest branches possess the usual three coats.

B. *The Maternal Placenta.*

The maternal elements of the placenta are developed from the uterine mucous membrane. The steps by which the normal mucous membrane is converted into the decidua have now been clearly made out. It must be remembered, however, that the fertilised ovum is not engrafted upon the mucosa in a state of quiescence, but as altered by the process of menstruation, that is, in a state of activity. It is now possible to give an outline, at anyrate, of the changes which occur in the uterine mucous membrane of the human subject during menstruation.

The earliest change in menstruation is hyperæmia and swelling of the mucous membrane, associated with hyperplasia of its connective tissue elements. The glands become dilated and contorted, and their epithelium proliferates; the connective tissue elements multiply, and, according to some, a marked infiltration of the stroma with leucocytes occurs. No trace is, however, found of the large decidual cells so well known in connection with pregnancy. Hæmorrhages occur in the superficial layers, but whether from actual rupture of capillaries is not made out; it is, however, certain that no considerable vessels rupture. In consequence of these hæmorrhages the superficial layers are broken up and cast off along with the covering epithelium. It is probably at this moment that the fertilised ovum, reaching the uterus becomes engrafted upon the altered mucous membrane.

This account, based upon observations on the human uterus, may be supplemented by the recent researches of Heape into the menstrual phenomena of *Semnopithecus entellus* (⁴⁸). In these monkeys Heape

has made out that a definite increase in size and number of the vessels occurs in the superficial layers of the mucosa, and that the dilated vessels, probably capillaries, rupture and form small hæmorrhages beneath the epithelium. Later, the extravasated blood collects in lacunæ, formed by destruction of the connective tissue, which in time burst through the epithelium, and allow the blood to escape into the uterine cavity. The rupture of the vessels is probably due to (1) thinning of their walls (2) diminished support of surrounding tissues, (3) some undetermined form of degeneration affecting their walls.

If a fertilised ovum does not reach the altered mucous membrane, the stage of activity subsides, and the damage done is probably soon repaired, the epithelial layer being regenerated from the glandular epithelium which remains. Under the stimulus of the presence of a fertilised ovum, however, the mucous membrane enters upon a new career, and becomes the decidua of pregnancy.

The changes which occur in the formation of the decidua may, for convenience, be divided into three stages:—

The first stage is characterised by the development of decidual cells, and the occurrence of extensive hæmorrhages in the mucosa. The decidua vera, reflexa, and serotina show the changes characteristic of this stage.

The second stage affects the serotina only. It consists in the invasion of the serotina by the chorionic villi, and the opening up of the maternal vessels, by which the circulation through the intervillous spaces becomes established.

The third stage represents the adult phase, in which the serotina consists of two distinct layers—a superficial compact, and a deep cavernous layer.

After the completion of the first stage the vera and reflexa undergo retrograde changes.

First stage.—In the first stage the decidua differs from the mucous membrane of menstruation in two essential particulars—first, in the presence of large numbers of large, round, nucleated cells, known as the decidual cells; and, secondly, in the widespread occurrence of dilatation and rupture of the vessels.

The decidual cells may be considered to be characteristic of pregnancy. It is certain that they do not occur during menstruation. Similar cells have, however, been observed by Overlach (⁴⁰) in acute phosphorus poisoning, and by Calderini (⁵⁰) as the result of experimental irritation of the uterus in animals. Pregnancy is, however, the only instance in which they occur under normal conditions. The characters of decidual cells are too well known to need recapitulation. Their development from connective tissue corpuscles has been repeatedly observed—Hart and Gulland (¹⁵), Minot (²²), Bumm (⁵)—and may be readily traced in a young ovum. The stages by which the spindle cell becomes the large round decidual cell are presented *ad naturam* in

Plate XIX. Fig. 1. Transition forms of every shape and variety may readily be found. They generally show a single, large oval, deeply-staining nucleus; at times there are two nuclei, very rarely three. In almost all parts red blood corpuscles are found lying singly or in heaps among the decidual cells.

The presence of giant cells in the decidua has been known for many years; they are generally supposed to be developed from decidual cells by a process of nuclear division. I am convinced that this is incorrect, and that the so-called giant cells are in reality sections of epithelial buds from the chorionic villi, which have become embedded in the decidua. This point will, however, be referred to more fully later on.

The small vessels are all markedly dilated, and their walls thinned. These changes are most marked towards the surface, where many vessels have ruptured, with extravasation of blood into the decidual tissue. The blood poured out breaks up the surrounding tissues, and finally makes its way out into the free space between the chorion and the decidua. Some of the hæmorrhages in the decidua are of large size, and often considerable extravasations into the intervillous spaces are found at the end of the first month. These changes, representing the first stage, are mainly preparatory, and are probably completed towards the end of the first month. They occur alike in the decidua vera, reflexa, and serotina; the second and third stages, however, are limited to the serotina.

Second stage.—The period during which the changes, characteristic of the second stage, occur cannot be exactly defined. They probably commence about the end of the first month; round the growing margin of the placenta they may continue until the last weeks of gestation, although in the more central parts they are completed earlier. They are in fact progressive and contemporaneous with the period of growth of the placenta itself.

There has been great difference of opinion expressed about the development of the serotina, and it is impossible, within the limits of this paper, to review the many theories that have been advanced. I shall, therefore, only refer to results which have been satisfactorily confirmed.

The changes which occur in the serotina are due to the remarkable activity of the chorionic villi. Wherever the placenta is growing the process of proliferation by budding, already described, goes on actively in the villi. These also invade the serotina, break up its tissues, and bore through the walls of its vessels. In this manner the maternal circulation through the intervillous spaces becomes established.

A section of the serotina at the beginning of the second month is shown in Plate XX. Fig. 5. The dilated and turgescient state of the vessels is very striking. Hæmorrhages into the tissue are seen at Hæm. At Gld. is shown a section of a dilated gland, well preserved, although detached, cubical epithelium; in many sections of glands the epithelium

is markedly proliferating. The decidual cells are loosely arranged, and in places there are masses of small round cells among them. The tissue is in parts extensively broken up by hæmorrhage; many of the glands contain blood (Plate XX. Fig. 6, Plate XXI. Fig. 11), and some of them are seen to communicate by a rupture with a neighbouring hæmorrhage (Plate XX. Fig. 6). In this manner irregular cavities full of blood are formed by rupture of vessels, and opening up of the surrounding decidual tissue.

In the neighbourhood of the hæmorrhages the decidual cells are seen to be actively engaged in absorbing blood (Plate XX. Fig. 6). Some of them contain only a small quantity of blood in the perinuclear protoplasm. Others are full of blood, and in these the nuclei are more deeply bloodstained than the protoplasm. The glandular epithelium may also, in the same way, be found absorbing the blood effused into the gland channel (Plate XX. Fig. 6). This process is probably an attempt at repair, on the part of these active cells. I am not aware that it has been described in the human serotina before.

It has been already stated that maternal blood becomes effused into the intervillous spaces as early as the end of the first month. This blood is to some extent reabsorbed by the chorionic epithelium (Plate XX. Fig. 6). The proliferating epithelium covering the villi, and the epithelial buds, are equally active in this respect. This process has been described by Strahl⁽⁴⁴⁾ as occurring in the placenta of the bitch. It has no nutritive significance, but is merely a method of disposing of what is practically a foreign body. There is no effective circulation through the intervillous spaces at this period, and blood effused into them represents practically an interstitial hæmorrhage.

Masses of small round cells are often found in the neighbourhood of the serotinal hæmorrhages (Plate XX. Fig. 5). Some of them are, no doubt, leucocytes, but very many arise from the decidual cells by a process of division. The transition stages of this process are represented *ad naturam* in Plate XIX. Fig. 2. These cells are probably concerned in the absorption of blood, and in the process of repair.

This is the condition in which the serotina is found at the period in which the villi begin to invade it. For many years the view first advanced by Turner (*loc cit.*) was maintained, that the villi penetrate the serotina by entering the open mouths of the dilated glands. It is now agreed that this is an error. Early in the second stage the mouths of the glands become closed, although the deeper dilated portions remain until the end of gestation. The villi bore their way directly into the serotinal tissues.

The process of penetration appears to depend upon the activity of the epithelial buds. In Plate XX. Fig. 6 are seen a number of sections of buds which have buried themselves in the serotina; the specimen is from the middle of the second month. By the steps which have been previously described, the bud becomes a villus, from which in

turn fresh buds arise. There is no evidence that fully formed villi push their way into the serotina by growth from behind. In this manner, first the superficial layers, and then the deeper ones, are invaded by the villi. Plate XX. Fig. 6 shows a deeply situated hæmorrhage in which a villus is embedded, and around it are numerous sections of epithelial buds, arising from other villi which do not appear in the section. Those parts of the serotina, which are thus invaded by the villi, become excavated; already disintegrated by hæmorrhage they now become entirely broken down, and thus pits or depressions are formed upon the serotinal surface.

This process of excavation results in the formation of an irregular series of elevations and depressions upon the surface of the serotina. It is found that the arteries open upon the elevations, while the veins open upon the intermediate depressions, Bumm (*), Ahlfeld (†). No one seems yet to have definitely proved that the arteries are opened up by the villi penetrating their walls. There remains, therefore, the possibility that the arterial capillaries rupture from the force of the blood current, assisted by thinning of their walls, and loss of support from surrounding tissues. An opening once made tends continually to enlarge by the action of the outflowing blood upon the loose decidual tissue. Villi cannot enter the channel against the blood current; the openings of the arteries are, therefore, always clear of villi, which are washed away from its immediate neighbourhood. The walls of the veins, on the other hand, are probably broken through by the villi. The direction of the blood stream favours their penetration, and venous channels can generally be distinguished by the fact that they contain sections of villi or epithelial buds. By the end of the second month there is probably a definite circulation through the intervillous spaces.

It is at this period that the so-called giant cells have been described as appearing in the placenta. I believe that these cells are, in reality, sections of epithelial buds. They have been described as present in all parts of the serotina; but especially in the deeper parts, and in the venous channels. It is in precisely these parts that the epithelial buds are most numerous. Structurally, the buds are merely multinucleated plasmodia, and so are giant cells. The only question is, therefore, as to their origin. After a very careful search I could find no evidence of the formation of giant cells from decidual cells, while their origin from the chorionic epithelium has been already demonstrated in Plate XIX. Fig. 4 and Plate XXI. Fig. 8. I am, therefore, inclined to believe that they are, in reality, foetal structures.

As a result of the excavation of the serotina, which occurs at this period, small portions of decidual tissue appear at times to become detached, and carried into the intervillous spaces by the blood current. They may even be found immediately beneath the chorionic membrane. They become moored to one or more of the villi, and later on undergo retrograde changes, which are of considerable importance in connection

with the formation of the white placental infarct. This matter does not, however, come within the scope of the present paper.

When the intervillous circulation has been established, no further active changes occur in the serotina at that part. Round the growing placental margin, however, changes characteristic of the second stage may be found up to the last weeks of gestation.

The process by which the placenta increases in size deserves a moment's consideration, and this is perhaps the most convenient place at which to refer to it. The growth of the placenta is intimately associated with the development of the decidua reflexa. It is now agreed that the reflexa is formed by a splitting of the vera around the base of attachment of the ovum. The superficial layer, containing numerous vessels and glands, grows up around the ovum and encloses it. The reflexa is, therefore, the superficial layer of the vera reflected over the ovum. It is nourished by vessels which enter its base from the vera. It is thickest in the parts nearest its junction with the vera, and towards the free pole of the ovum it thins gradually away; this thinning being probably brought about by the pressure of the growing ovum, and the distance of the part from the source of blood supply. As the ovum grows the reflexa is carried with it and thus becomes raised from the vera over a continually increasing area. In this manner room is provided for the peripheral growth of the placenta. This growth is at first very rapid. At the end of the second week the base of attachment of the ovum is equal in area to about one-fifteenth of the total uterine surface. By the end of the third month the placenta occupies one-fourth of the total uterine surface, and this proportion is maintained up to the end of pregnancy. That is to say, after the third month, the growth of the placenta is only proportional to that of the entire uterus. After the third month, too, the functions of the reflexa cease to be of importance; the growth of the ovum has overtaken that of the uterus and fills it entirely, and the reflexa atrophies from constant pressure.

Glycogen has been found by Merttens ⁽³¹⁾ and others in the glandular epithelium and in the decidual cells of the serotina. It is contained in fairly definite areas of the periphery of these cells. The presence of glycogen in the placenta was discovered by Claude Bernard ⁽⁵¹⁾, but I am not aware that it has been precisely localised before. I have not been able to find glycogen in any of my specimens, and I am, therefore, inclined to believe that its presence is not constant.

Third stage.—By the time that the intervillous circulation is established the placenta has assumed its adult form. The serotina now undergoes some measure of consolidation and repair. The hæmorrhages become to a great extent cleared up, although blood corpuscles may in parts be found among the decidual cells at the mid-term of gestation. The decidual cells become more closely packed, forming an altogether denser type of tissue than that of the earlier stages. Villi are unable

to penetrate it for any considerable distance, but they become attached in large numbers to its surface.

Even at the beginning of pregnancy the decidua is roughly divisible into two layers: a superficial layer, in which the hæmorrhages are most numerous, and a deep layer containing the dilated glands. In the third stage, however, the serotina consists of two perfectly distinct layers: a superficial compact layer, and a deep cavernous layer (Plate XXI. Fig. 12). *The superficial layer* is composed of closely-packed decidual cells, and is traversed by the placental arteries and veins, which open upon its surface into the intervillous spaces. There are no glands in this layer. The surface is covered by a stratum of fibrin. *The deep or cavernous layer* contains a network of irregular, dilated, glandular channels. In the third and fourth months, many of the glands retain a well-preserved lining of cubical epithelium. Towards the end of pregnancy, only occasional small groups of these cells remain; so that the channels are often mistaken for veins. The arteries and veins, of course, also pass through it. The decidual stroma consists of cells smaller and more closely packed than those of the superficial layer. It is through the deep layer that separation of the placenta occurs when it is normally shed.

The course of the maternal vessels deserves more detailed notice. The fact that these vessels opened into the intervillous spaces was proved long before the details of the process by which this communication becomes established were worked out. Waldeyer⁽⁴³⁾ first succeeded in making satisfactory observations upon this point, and his research is one of the landmarks of the study of the placenta. Very little has since been added to his description of the course of the maternal vessels in the adult placenta.

There are very few capillaries in the serotina at this period; the arteries and veins open directly into the intervillous spaces, but before doing so they give off a few nutritive branches to the serotina. The arteries run a spiral course right through the uterine wall. In many naturally shed placentæ, the torn ends of these arteries may be detected upon the maternal surface. In the muscular wall of the uterus, they show the usual three coats, and round many of them is found a semi-circular, peri-lymphatic space. When the artery leaves the muscular wall the middle coat disappears, and in the serotina there is only an endothelial layer surrounded by a loose fibrous sheath. The artery enters the serotina vertically, but alters its course so as to open obliquely into the intervillous spaces. The fibrous sheath ceases abruptly just before the artery opens. Waldeyer asserts that the endothelial layer may be traced not only up to the opening, but for some distance over the surface of the adjacent serotina. Others believe that it disappears with the fibrous sheath, so that the actual aperture is formed only by the rounded edges of the decidual tissue (Plate XXII. Figs. 15 and 16).

The veins form large sinuses in the muscular wall of the uterus.

On reaching the serotina their course is nearly parallel to its surface, they then rise gradually and so open very obliquely into the intervillous spaces.

We are now in a position to refer to the vexed question of the origin of the intervillous spaces. In the first place, the boundaries of the general intervillous space should be pointed out. Towards the uterus it is limited by the serotina; towards the fœtus by the chorionic membrane, from which the villi arise; at the placental margins by a reflection of the serotina beneath the chorionic membrane. The placental margin represents the line of union of the three parts of the decidua; the decidual tissue is here especially thick, it supports the circular sinus and contains numbers of embedded villi. It is prolonged into the placenta beneath the chorionic membrane for 1 to 2 inches, thinning out gradually as it passes inwards.

The question at issue has been, Are the intervillous spaces altered maternal vessels or not? The solution has been sought in the direction of determining whether the spaces are entirely enclosed in tissue of maternal origin, that is, whether or not the villi receive an investment of maternal tissue. Among the earliest advocates of the view that the intervillous spaces are maternal were Turner and Ercolani. They based their theory upon the opinion they had formed that the villi of the adult placenta possessed a complete investment of maternal tissue; the intervillous spaces could then quite properly be regarded as altered maternal blood vessels. They did not attempt, however, to establish their views by tracing the development of these spaces. Somewhat the same theory has been revived in a different form by Waldeyer, and also by Keibel. These observers believe that they have found a delicate layer of endothelium covering the villi, which they hold to be the representative of the walls of dilated maternal capillaries. Keibel's observations (¹⁸) are so incomplete that little importance can be attached to them. Waldeyer's observation, therefore, stands alone, and it is important to remember that it derives no support whatever from the facts which have been established in regard to the beginnings of the placenta. More recently still, Kossmann and Merttens (*loc. cit.*) have attempted to return to the theory of Turner and Ercolani by applying the facts of comparative anatomy to the human placenta. Reasons have, however, already been given for suspending judgment upon this point for the present.

The methods by which these theories have been arrived at are open to objection. The question cannot be solved by observations upon the adult placenta (that is, upon the placenta in the second stage), supplemented by theories accounting for what is then found. The facts can only be arrived at by tracing in detail the steps by which the intervillous spaces are formed; when this is satisfactorily accomplished their origin is no longer a matter of theory but of fact. An attempt is made in this paper to describe the changes in the placenta which result in the formation of these spaces. These changes occur, however, at a very

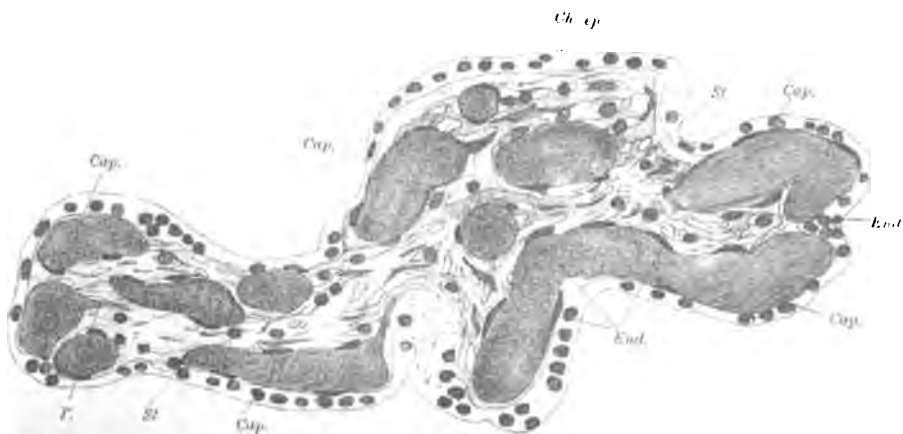
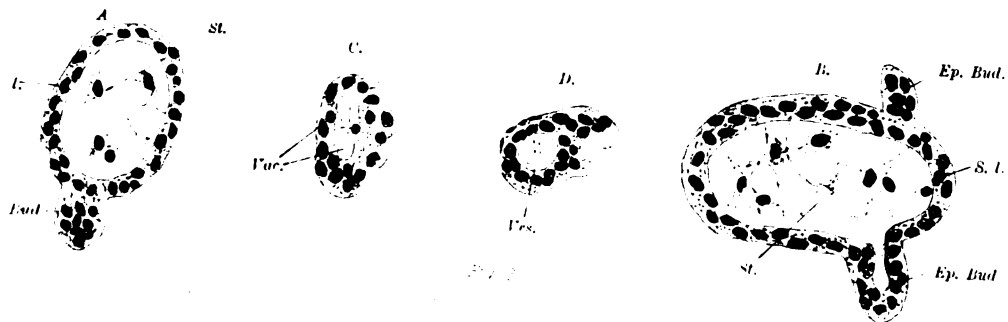
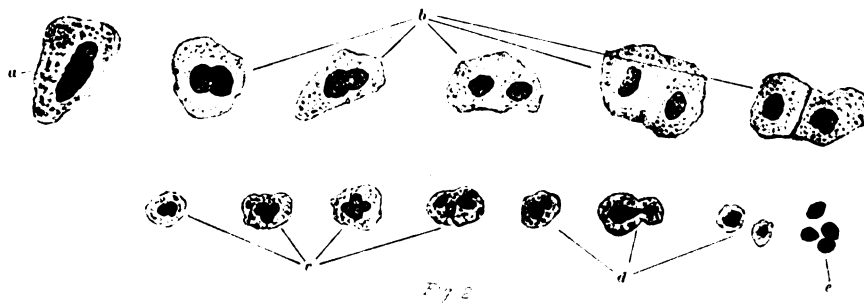
early period when it is difficult to obtain material in a condition suitable for examination, and further observations are, no doubt, needed. But if the account given here be correct, then the intervillous spaces are not dilated maternal capillaries, nor are they spaces enclosed in maternal tissue at all; they are made up as follows:—

1. By the space primarily existing between the decidua and the chorion;
2. By spaces formed by destruction and absorption of decidual tissue;
3. By glandular and vascular maternal channels, opened up in the same way.

In the spaces thus formed the villi develop, and many of them become attached to the serotina. They are in direct contact with the maternal blood, which circulates among them. This arrangement seems in all respects admirably adapted to promote osmotic interchanges between the foetal and maternal blood, the chorionic epithelium representing the dialyser. This osmotic interchange is the essential condition of the nutrition and development of the foetus. Very few facts, however, are known regarding the details of the process.

The circulation through the intervillous spaces is probably not a rapid one. This is an advantage in giving time for the occurrence of the osmotic changes. The spiral course of the arteries tends to considerably reduce the force of the blood stream, a modification which is probably necessary in view of the delicacy of the placental structures. The outflow from the spaces is chiefly promoted by the intermittent uterine contractions, which have the effect of aspirating the contents into the veins.

During the third stage villi are always found attached to the surface of the serotina, and some are embedded in it. The embedded villi are devascularised and functionless. When a villus becomes attached to the serotina, it loses first its superficial epithelial layer along the line of contact. The cells of the deep layer are then brought into contact with the decidual cells, and a marked proliferation of the deep layer occurs, so that a considerable area of round cell infiltration is formed around the site of attachment (Plate XXII. Fig. 13). This has the effect of welding the villus to the serotina. The process was first described by Langhans⁽²⁷⁾, and he regarded it, as it seems to me, rightly, as evidence of the mesoblastic origin of the cells of the deep layer of what is called the chorionic epithelium. At times the proliferation of the deep layer is so extensive as to form a considerable layer of round cells lying upon the surface of the serotina. The superficial layer of the chorionic epithelium is generally prolonged from the sides of the attached villus over the adjacent serotinal surface for a short distance, but it does not form anything like a continuous layer (Plate XXII. Fig. 14). The death of the embedded villi at this period is due to the fact that they find, in the compact decidual tissue, none of those condi-



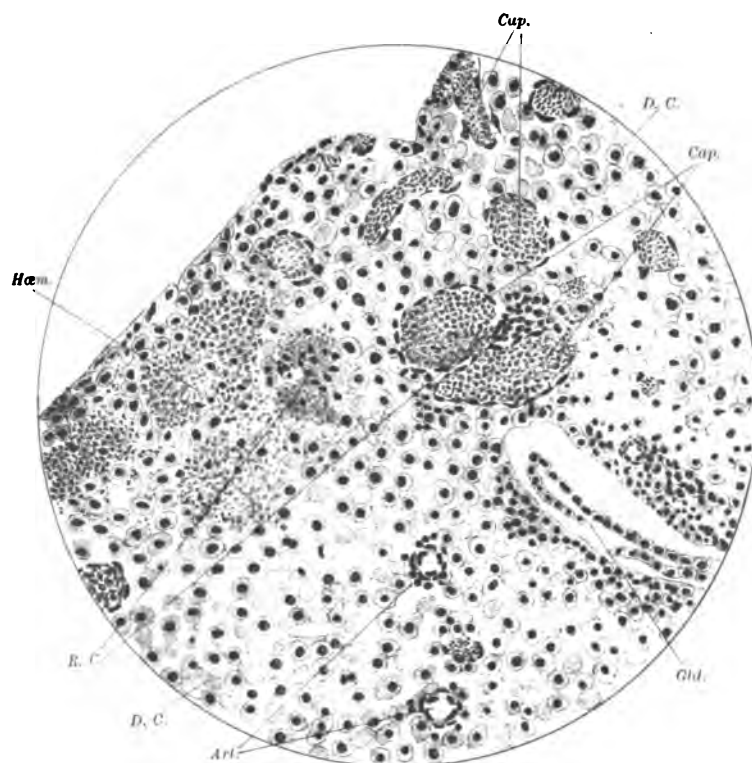


Fig 5

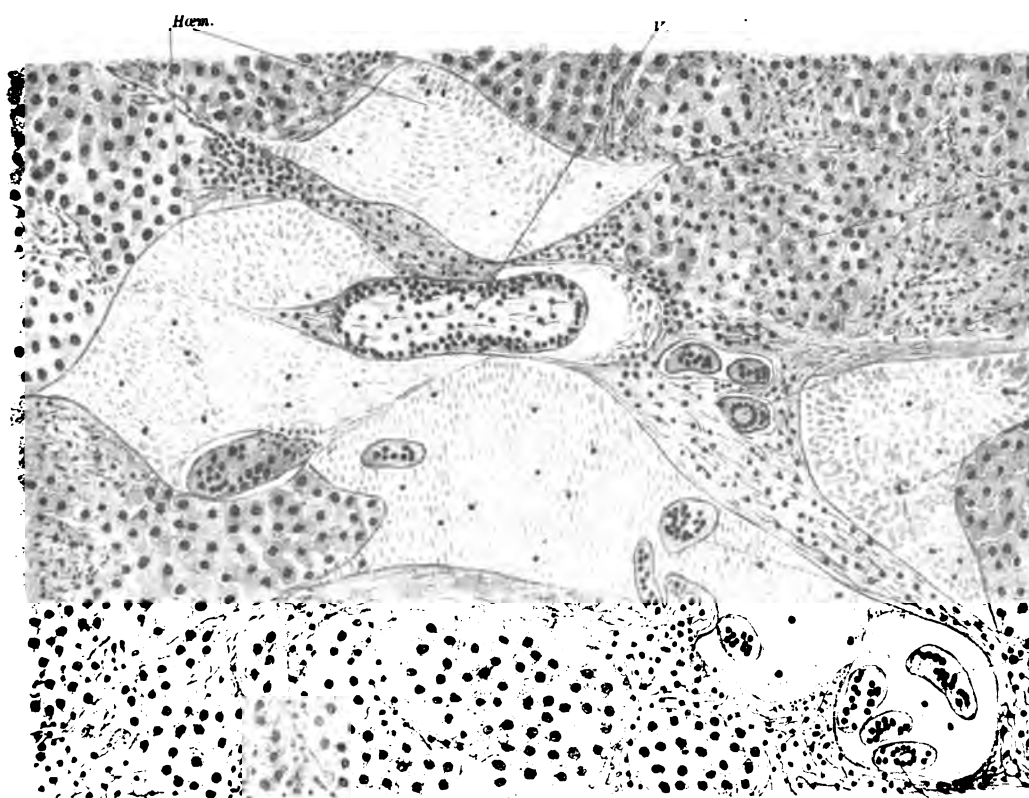




FIG. 7.



FIG. 8.



FIG. 9.

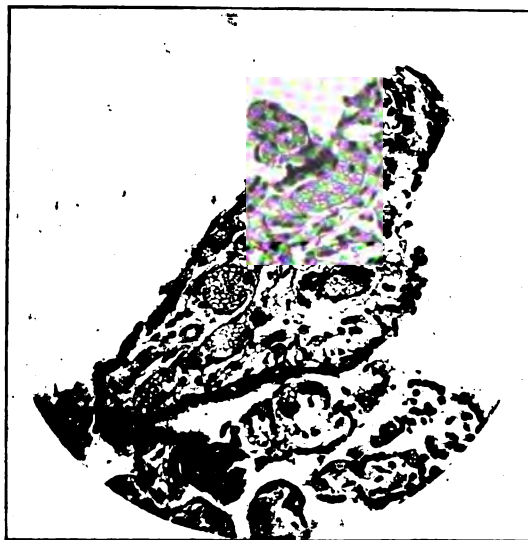


FIG. 10.

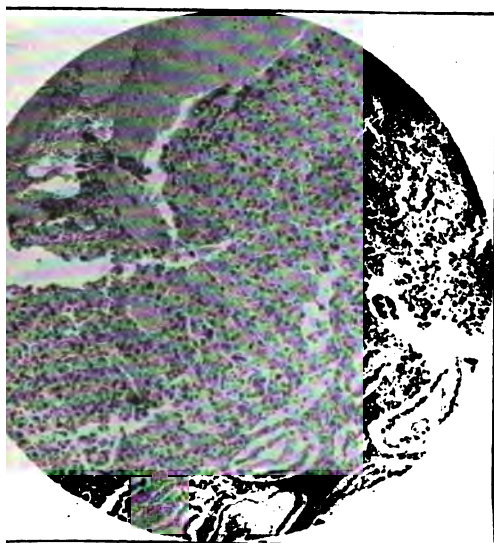


FIG. 11.

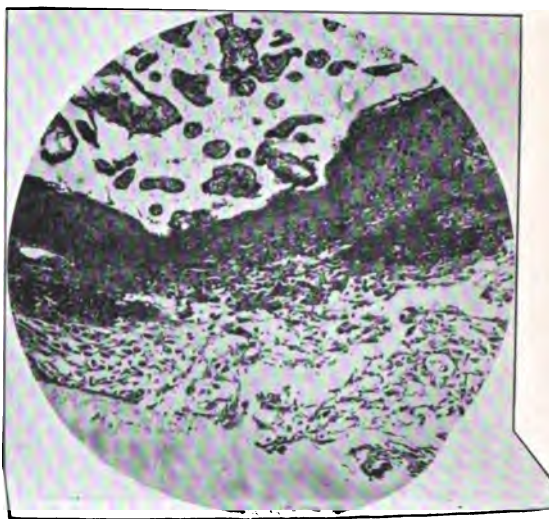


FIG. 12.

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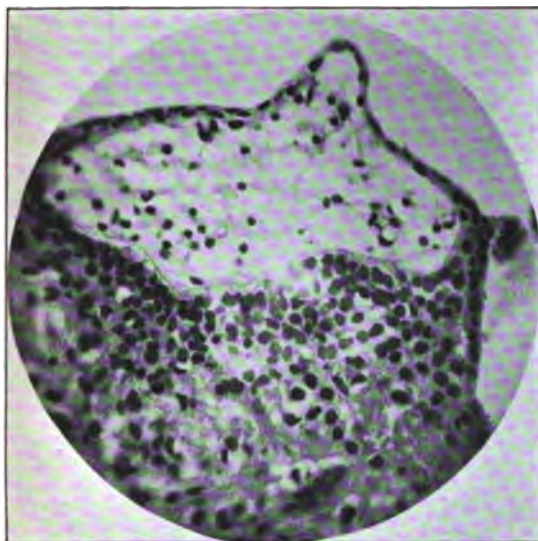


FIG. 13.

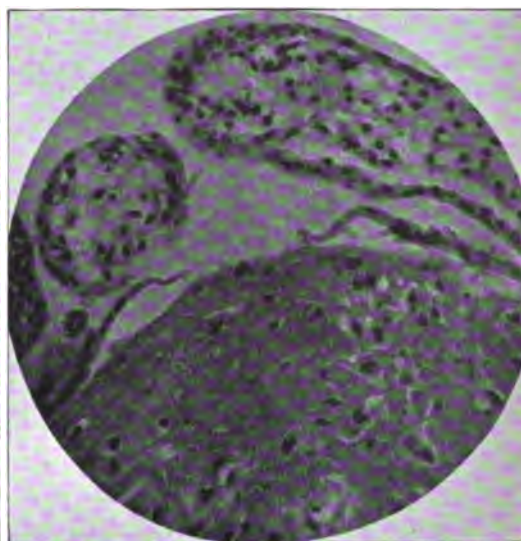


FIG. 14.

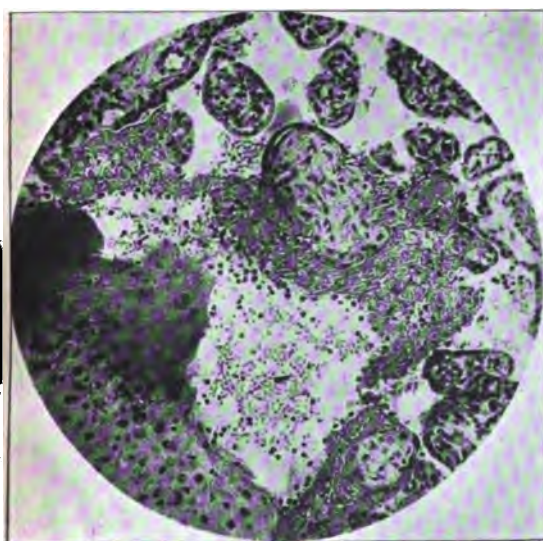


FIG. 15.

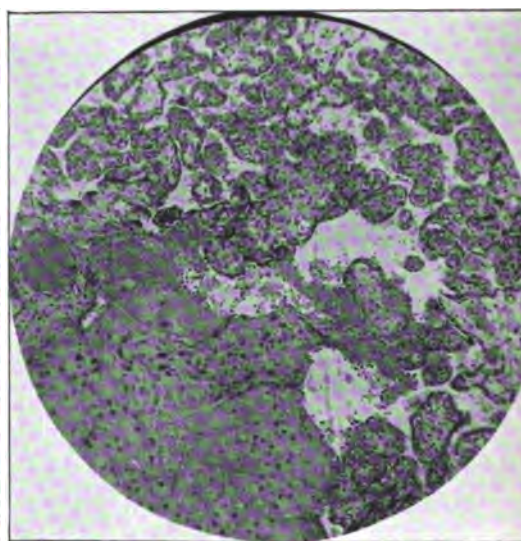


FIG. 16.

early period when it is difficult to obtain material in a condition suitable for examination, and further observations are, no doubt, needed. But if the account given here be correct, then the intervillous spaces are not dilated maternal capillaries, nor are they spaces enclosed in maternal tissue at all; they are made up as follows:—

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tions essential to their nutrition which are present in the earlier stages.

I have endeavoured in this paper to deal only with those changes and processes by which the human placenta is carried to its highest point of development and functional activity. The placenta at term is an overripe and decadent structure. Many changes are found in it which can only be regarded as degenerative, and these are left over for consideration in a subsequent paper. Among the matters thus left over, it is perhaps necessary to mention the subject of the origin of the substance known as canalised fibrin, which plays such an important part in the structure of the placenta at term. I believe the appearance of this substance to be the direct result of degenerative changes, and it has therefore not entered into the scope of the present paper. It is referred to now merely to avoid misconception.

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DESCRIPTION OF PLATES XIX. TO XXII.

PLATE XIX.

FIG. 1.—Represents the development of decidual cells from connective tissue corpuscles. The different forms are all drawn from the section represented in Figs 6 and 7. (Leitz, Oc. 3, Obj. 7.)

- a. Connective tissue corpuscles.
- b. Intermediate forms.
- c. Decidual cells.

FIG. 2.—Represents the formation of small round cells from decidual cells by fission. (Leitz, Oc. 3, Obj. 7.)

- a. Decidual cells.
- b. Cells in various stages of division.
- c. The resulting small round cells, many of them closely resembling leucocytes.
- d. Further division of the small round cells.
- e. Free nuclei.

FIG. 3.—Illustrates the process of budding and formation of new villi. The sections are from a complete ovum at the sixth week. (Leitz, Oc. 3, Obj. 7.)

A. Section of villus.

S.L. Superficial layer of the chorionic epithelium; the deep layer is not seen, it is often absent in young villi.

Ep. bud. Epithelial bud, springing from the superficial layer.

St. Stroma of villus.

B. Section of a rather larger villus than *A*. Two epithelial buds are here shown; into the lower one the connective tissue stroma is prolonged.

C. Horizontal section of an epithelial bud. There are two vacuoles present, and the nuclei are arranged roughly round the periphery.

D. Horizontal section of a bud further developed than *C*. There is a section of a vessel near one end, with a distinct wall, and containing blood in its lumen.

FIG. 4.—From a placenta of the seventh month, injected with carmine-gelatine, by means of a tube and funnel. The section is stained with logwood. (Leitz, Oc. 3, Obj. 7.)

Cap. Capillaries filled with injection; they run for the most part immediately beneath the epithelium of the villus.

St. Stroma of the villus saturated with the injection; the nuclei and the protoplasmic network are uninjured, the injection being contained in the meshes.

T. Small twig springing from a capillary, and opening into the meshes of the network.

End. Endothelial cells representing the capillary wall.

PLATE XX.

FIG. 5.—Vertical section through the decidua serotina at the sixth week. From the same specimen as Fig. 3. (Leitz, Oc. 3, Obj. 3.)

D.C. Decidual cells.

R.C. Clusters of small round cells.

Cap. Distended capillaries, some of which have ruptured.

Hæm. Hæmorrhage into the decidual tissue.

Gld. Section of a gland, with cubical epithelium.

Art. Arterioles.

FIG. 6.—Shows the penetration of the villi into the decidual tissues. From the same specimen. (Leitz, Oc. 3, Obj. 3.)

D. Decidua.

V. Section of a villus embedded in a hæmorrhage.

Hæm. Hæmorrhages.

Ep. buds. Sections of epithelial buds from other embedded villi, not shown in the section.

(a) (a). Buds showing sections of vessels in their interior.

PLATE XXI.

FIG. 7.—From a three months' placenta, showing the two layers of the chorionic epithelium, and the protoplasmic network of the villus stroma.

FIG. 8.—From a six weeks' ovum. Villus showing the proliferation of the chorionic epithelium and an elongated epithelial bud.

FIG. 9.—From a six weeks' ovum. Shows the epithelial covering of the chorionic membrane, which is budding in places, and the proliferation of its nuclei. Two large epithelial buds appear in section, one of which contains two vessels, the other, one.

FIG. 10.—From a nine months' placenta, in which the foetal blood had been retained (natural injection). The villus in section shows several large capillaries packed with blood, and many sections of smaller ones scattered through the stroma.

FIG. 11.—From a six weeks' ovum. The section is through the deeper part of the decidua serotina. Several glands are shown, containing blood, in the lower part of the figure; a large hæmorrhage occupies the upper part. At the margin of the hæmorrhage is a large epithelial bud with two vessels in section.

FIG. 12.—From a seven months' placenta. The figure shows the two layers of the serotina at this period; the dark band along the surface is composed of canalised fibrin.

PLATE XXII.

FIG. 13.—From a three months' placenta. Shows a section through an attached villus, and the adjacent serotina. Along the line of attachment the epithelial covering is indistinct; its nuclei have proliferated markedly, and invaded the adjacent decidual tissue. At the right of the figure the superficial layer of the epithelium is seen to be prolonged for a short distance upon the serotinal surface.

FIG. 14.—From the same placenta. Shows the serotinal surface with an interrupted layer of chorionic epithelium upon it.

FIG. 15.—From the same placenta. Shows a maternal vessel in section, on the point of opening, at the apex of a decidual elevation, into the intervillous spaces.

FIG. 16.—No. 4 of the series of sections, of which Fig. 15 is No. 1. Shows a point where the maternal vessel has opened in two places into the intervillous spaces.

ON A CASE OF MULTIPLE FOCI OF INTERSTITIAL MYOCARDITIS IN HEREDITARY SYPHILIS.

By LUDWIG HEKTOEN, M.D., Chicago.

From Prof. Chiari's Pathological Institute, Prague.

ACQUIRED syphilis not unfrequently produces changes in the heart; in hereditary syphilis, on the other hand, the heart very rarely seems to be involved, and the number of cases of hereditary syphilitic heart lesions described in the literature is exceedingly small.

No statistics bearing upon this point seem to exist. Petersen¹ found syphilitic heart changes ten times in a statistical material of 88 cases of mostly acquired syphilis, but he makes no attempt to consider separately hereditary heart syphilis.

Mueller² examined the bodies of 18 syphilitic infants, but he makes no mention of having observed any changes in the heart.

In the recent study of heart syphilis by Mraček³ are collected, from the literature of the subject, 112 cases of this lesion, but of this number only 9 (von Rosen, Williams, Wagner, Morgan, Kantzow Virchow, Money, Shattock, Coupland, Orth) are described in connection with hereditary syphilis, and the luetic nature of some of these is certainly very doubtful, to say the least. Mraček, furthermore, studied the material furnished by 150 post-mortem examinations of congenitally syphilitic infants, and the outcome, so far as the heart is concerned, consists of four examples of congenital syphilitic myocarditis, two being mentioned as representing a nodular or more gummatous form, the other two illustrating a more diffuse, acute, interstitial variety.

Since the publication of Mraček's work, it has not been possible to find any further cases in the accessible literature of the last year or two.

Partly in order to add a new case to the meagre literature of hereditary syphilitic heart lesions, partly in order to show in a strik-

¹ "Versuch einer path.-anat. Statistik der visceralen syphilis," *Monatsh. f. prakt. Dermat.*, Hamburg, 1888, s. 109.

² "Beiträge zur pathol. anatomie der syphilis hereditaria bei Neugeborenen," *Virchow's Archiv*, bd. xcii. s. 532.

³ "Die Syphilis der Herzene bei erworbener und ererbter Lues," *Arch. f. Dermat. u. Syph.*, Wien, Ergänzungsheft ii. 1893.

ing manner the remarkable extent that such processes may reach, it has been thought advisable to publish the following instance of interstitial myocarditis in a luetic infant, the organs of which were kindly placed at my disposal for microscopic examination by Professor Chiari, whom I hereby thank for his advice during the preparation of this report.

The case was that of a female child 6 weeks old, from the clinic of Professor Ganghofner, which showed, when 4 weeks old, a macular exanthema that soon developed into pemphigus; at the same time diarrhoea and exhaustion set in, and rapidly caused death. It was the first child, and there was no history of previous abortions. The clinical diagnosis was hereditary syphilis.

POST-MORTEM EXAMINATION.—The body is 45 cm. long, of feeble build, and quite emaciated. The skin is everywhere covered with small, dried vesicles or brownish crusts. On the back there are a few pale livid patches. The rigor mortis is well marked. The hair is brownish, thick; the pupils equal and slightly dilated. The neck is short and thin; the thorax long and broad. The abdomen is of normal dimensions. The scalp is anæmic; the skull, 35 cm. in horizontal circumference, is normally ossified; the dura is everywhere adherent to the calvaria; in the dural sinuses there is dark red, fluid blood. The pia and the brain are without any changes. The diaphragm reaches, on the right side to the upper, on the left side to the lower, border of the fifth rib.

The mucous membrane of the mouth, pharynx, and larynx is normal. The thymus and thyroid glands are of proportionally normal size. The lungs are partly distended, partly collapsed, dark red in colour, firm in consistence; a small amount of yellow muco-pus can be squeezed out of the bronchi.

In the pericardium there are no abnormal contents, and the layers are smooth and shining.

The heart corresponds fairly in size relatively to that of the body; the myocardium is pale, and the walls of the ventricles are somewhat thickened, the right measuring 6 mm., the left 11 mm., and the ventricular septum 7 mm., in thickness. The left ventricle is 3.5 cm., the right 3 cm., in depth. In the walls of the ventricles are numerous whitish, round areas, which are quite sharply circumscribed, and cause slight bulgings of the surface here and there; the cut surface of these areas is of a homogeneous appearance; there are no signs of any disintegration, they are not calcified, and there is no encapsulation; they cut with perhaps a little more resistance than the normal-looking heart muscle. These areas vary very much in size, the largest measuring 1 cm. They occupy in about the same number the anterior and posterior walls of both the ventricles, and are distributed thickly throughout the interventricular septum; they bear no constant relation to the vessels. The auricles do not show any changes.

The macroscopic coronary arteries appear healthy.

The heart valves are thin and smooth, and there are no developmental defects or anomalies in the heart or the larger vessels.

The liver is quite large, yellowish-brown, of homogeneous appearance on the cut surface.

The spleen is also somewhat enlarged, quite firm, partly deep red, partly brown in colour.

The kidneys are normal in size, light grey in colour, of usual consistence, and without any naked-eye changes.

The ureters, bladder, and genital organs are normal.

In the stomach there is a small amount of brownish fluid, the mucous membrane is pale. The mucous lining of the ileum is a little reddened and swollen.

The pancreas and the adrenals show no changes.

The line of ossification in the lower line of the femur is not quite sharp and straight, but rather broad and yellowish.

Anatomical diagnosis.—Congenital syphilis.

(Exanthema, enlargement of liver and spleen, gumata of the heart, osteo-chondritis.)

Catarrhal bronchitis.

Partial pulmonary atelectasis.

Acute catarrhal enteritis.

MICROSCOPIC EXAMINATION.—*The heart.*—Vertical segments, running from base to apex, were cut out of the right and the left halves of the

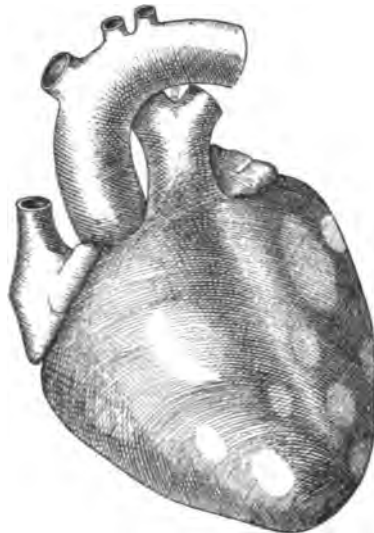


FIG. 1.—The anterior surface of the heart. The lighter areas show the foci of interstitial myocarditis.

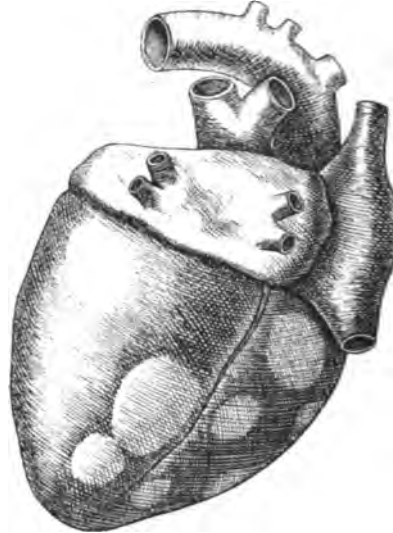


FIG. 2.—The posterior surface of the heart. The lighter areas show the foci of interstitial myocarditis.

anterior wall of the right ventricle, of the anterior and the posterior halves of the left margin of the left ventricle, and of its posterior wall, and of the anterior and posterior halves of the interventricular septum.

The whitish foci seen upon these sections from the heart walls were indicated upon diagrammatic outlines, in order to control the topographic relations of the possible microscopic changes. It was found that without any exception the microscopic changes, about to be detailed, corresponded exactly to the whitish, more or less circumscribed areas, noted in the naked-eye description.

The changes consist in an extensive infiltration into and between the muscular bundles, with rounded and oval nucleated cells, usually embedded in a fibrillated stroma. This infiltration occurs, as indicated, in quite large, fairly-circumscribed districts, and seems to proceed in the main from the adventitia of the smaller vessels, immediately around which the proliferation is usually most marked, and from which more or less distinct strands of young connective tissue pass into the adjacent tissue. The larger sub-

epicardial vessels are not involved; it is only the finer branches in the heart muscle that are concerned in these changes; the vessels are nearly always empty and contracted; there is no proliferation of endothelial lining, and the vessel walls are rarely infiltrated, but the adventitia contains more and denser fibrous tissue than is found in normal hearts from young infants examined for the purposes of control. The media of the smaller arteries is also considerably thickened.

The muscular fibres in the area of proliferation show an indistinct striation, the nuclei are usually distinct, but in many places the infiltration separates individual fibres, and in such it is usually difficult to recognise the typical structure. Only in one or two places,—and these were in the interventricular septum,—did the proliferation appear in the form of a distinctly nodular accumulation of cells; in one of these the centre showed an ill-defined degeneration, with appearances that reminded one of a necrotic giant cell, otherwise the proliferation is diffuse, but confined to the districts indicated in the drawings. There is no distinct fibrinous exudate in the changed areas, as no fibrin can be found in sections stained according to Weigert's fibrin method; neither are there any leucocytes with polymorphous nuclei.

The epicardium is not changed. The endocardium shows, very distinctly, accumulations of endothelial cells, in the recesses between the papillary muscles.

In the *lungs* the bronchi contain exudate with leucocytes and desquamated epithelial cells. In places there is collapse of the parenchyma with hyperæmia.

The *liver* has much increase of connective tissue in Glisson's capsule, and also a marked intralobular interstitial proliferation.

In the *spleen* there is dilatation of the venous sinuses, and some increase in the lymphoid elements.

The *kidneys* show no changes.

In the *lower* end of the *femur* there is a minimum degree of irregularity in the line of ossification.

REMARKS.—In the foregoing case we have an instance of multiple areas of interstitial myocarditis in a syphilitic child. The diagnosis of syphilis is based, not only upon the clinical manifestations, but also upon the firmest anatomical basis, to wit, the cutaneous eruption, the connective tissue proliferation in the liver, the splenic tumour, and the osteo-chondritis.

In the *heart* the interstitial changes were so marked, and so sharply defined, that it led to the anatomical diagnosis of gummata of the heart; in fact, the microscopic examination shows that the centres of some of the areas present the changes characteristic of gumma. This case is consequently one of nodular, or gummatous myocarditis in hereditary lues; as additional instances of this form of congenital syphilitic heart lesion, reference may be made to two cases described by Mraček.¹

While the clinical history of this case points to the cachexia, and the intestinal derangement as the causes of death, it cannot be denied that such an extensive focal myocarditis as has been described must in itself be a most dangerous lesion, in consequence of which death might ensue at any moment, no matter what the general condition of nourishment might happen to be.

¹ *Loc. cit.*

In fact, the case of congenital syphilitic myocarditis described by Coupland,¹ and one of the cases of acute exudative myocarditis in congenital syphilis described by Mraček,² both show sudden death in two children, that had previously been regarded as in good general health. In both these cases the changes in the myocardium were more acute and more diffuse than in the case here described; they were also accompanied by exudation of serum and fibrin.

It is evident that the dangerous nature of congenital syphilitic myocarditis, including, as shown, the liability to sudden death in apparently healthy children, adds an element of practical and clinical interest to such lesions, which is of much greater weight than that which they have previously possessed as mere curiosities in pathological anatomy.

¹ "Specimens from a Case of Infantile Syphilis, Interstitial Myocarditis, and Nephritis, Gummata in the Liver and the Lung," *Trans. Path. Soc. London*, vol. xxvii. p. 303.

² *Loc. cit.*

Note.—There is no ground for the assumption ventured by Mraček that Coupland's case is one of congenital malformation with consecutive hypertrophy of the heart. There is nothing in Coupland's description to indicate any defect in the ventricular septum, as Mraček suspects. In another place, Mraček speaks of Coupland's case as a possible gumma of the heart, whereas it is as pure and evident an instance of congenital syphilitic myocarditis as any on record.

AN UNCOMMON FORM OF TUMOUR OF THE THYROID BODY.

By F. VILLY, *Student of Medicine.*

From the Pathological Laboratory, Owens College, Manchester.

BEFORE describing in detail the case of malignant tumour of the thyroid gland which forms the basis of this paper, a short consideration of malignant thyroid tumours in general may not be out of place.

Taking all the species of such growths together, their rarity may be judged from the fact that the clearly malignant cases reported in the *Transactions of the Pathological Society of London* do not number twenty. To these may be added four, of which the description is not sufficient to enable one to decide with enough certainty as to the exact nature of the growth to justify their inclusion under any title.

To take these cases in a little more detail, five were carcinomata. These were all examined microscopically, and consequently there can be little doubt as to their nature. These cases seem to show that secondary deposits do not occur readily in thyroid cancer, for in two there were no such deposits, in three the lymphatic glands were infected, whilst in two only were metastatic deposits present—in one case in the lung, in the other in the kidney.

Of the remaining cases six were sarcomata. In only four of these is there a satisfactory account of the microscopical examination. In three there was no secondary deposit; in two lymphatic glands were infected; in one metastasis took place to the pericardium and heart; and in one to the kidneys; whilst another is interesting from the presence of an enlarged (not sarcomatous) accessory thyroid body.

These eleven cases are interesting mainly for their rarity. Four more are to be noticed as belonging neither to a carcinomatous nor a sarcomatous type, though they were undoubtedly malignant. They can only be classed as malignant adenomata.

Three of these four cases belong to the species in which the enlarged gland and the secondary growths repeat the normal structure;¹ in the remaining one the epithelium, though not assuming a carcino-

¹ Cohnheim, Virchow's *Archiv*; Morris, *Trans. Path. Soc. London*, 1880; Coats, *ibid.*, 1880; Howard, *ibid.*, 1882.

matous type, had proliferated so as to form intracystic papillomatous projections; along with this enlarged lymphatic glands were associated.¹

It is to this last class, apparently intermediate between the more simple form of adenoma and carcinoma, that the tumour to be described belongs. Such tumours have been previously described under the title of carcinoma; as will be seen, this name is not strictly correct, and its use has apparently led to lack of attention to the existence of this particular class of thyroid adenomata.

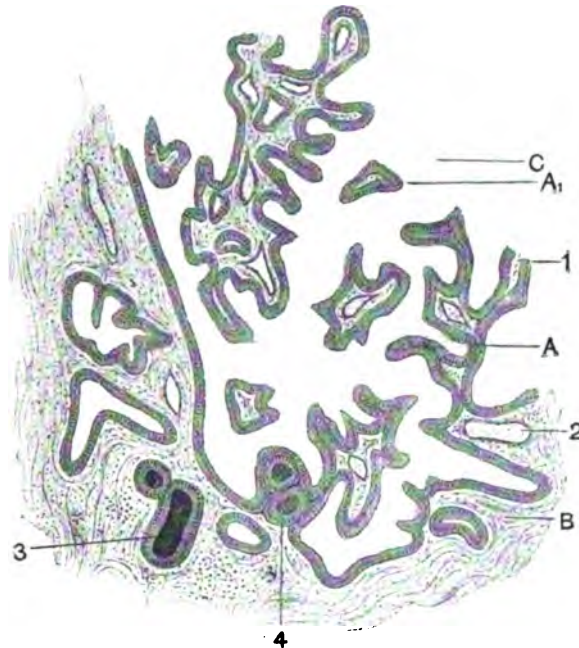
The patient, an Icclander, æt. 45, suffering from the tumour, was admitted to the Manchester Royal Infirmary, on 19th November 1894. He was under the care of Mr. Jones, to whom I am indebted for permission to describe the case. About a month previous to the date of admission he had first noticed a swelling on the left side of his neck; it was then about 1½ in. across. At the time of admission this had increased to about the size of a Tangerine orange; this increase had caused no pressure effects to appear in any neighbouring parts, nor was there any apparent emaciation. The growth implicated the left lobe and isthmus of the thyroid gland, and in physical characters was indistinguishable from an ordinary non-malignant bronchocele.

At the operation which was undertaken a median incision was made and the tumour exposed. This tumour was found to be cystic in its anterior part, and on incision a fluid described as "grumous" escaped. The posterior part was firmer though friable, and it projected into the anterior cyst as a large number of fine papillæ. The tumour as a whole was not encapsuled, but adherent to the neighbouring structures, especially in its posterior and left aspects. In spite of this it was successfully removed, and along with it three oval bodies, which were taken to be enlarged lymphatic glands; of these, two lay in a line close beneath the enlarged left lobe of the thyroid, the third being placed further down under the upper border of the sternum, from which position it was scooped out. These glands apparently belonged to the deep cervical series. The wound was entirely closed, and the patient made a good recovery, being discharged satisfied on 6th December. After the operation special investigation as to the presence of secondary deposits were made; no evidence of their existence was found in the viscera, bones, or any other part of the body, as far as could be ascertained by clinical examination.

Parts of the growth and glands so obtained were hardened in alcohol, other parts in Müller's fluid. These were embedded in paraffin by the chloroform method, sections cut, fixed to the slide, then stained with hæmatein and double stained with rubin and orange, a method of stain-

¹ Bilton Pollard, *Trans. Path. Soc. London*, vol. xxxvii.; Luigi Griffini, "Sur le developpement de l'Epitheliome du Corps Thyroide," *Arch. per le sc. med.*, Torino, vol. iv.; Cornil, *Arch. de physiol. norm. et path.*, Paris, 1875, p. 659.

ing usually adopted in the pathological laboratory. The tumour was then seen to be composed almost entirely of villus-like series of processes, projecting in part into the anterior cyst, and separated from one another by very irregular spaces. The deeper parts are more solid than those near the anterior cyst, and contain a certain number of small spaces having almost exactly the characters of a normal thyroid gland, including the presence of colloid matter. Taking one of the villi, its characters



Section through part of the walls of the cyst described.
(Slightly diagrammatic.)

- A. A papillary growth cut longitudinally.
- A₁. A papillary growth cut transversely.
- B. Fibrous stroma.
- C. Cavity of the cyst.
- 1. Epithelial lining of the cyst.
- 2. Blood vessel in the stroma.
- 3. Cyst containing colloid matter.
- 4. Small diverticule containing colloid matter.

may be studied more closely. The immediate outer coating, abutting on the cyst contents, consists of a clearly marked single layer of columnar epithelium; the cells of this layer are not very distinctly separated from one another, but the oval nuclei are shown very plainly. In no section examined, and these are numerous and taken from several parts of the growth, have any signs of an arrangement of the epithelial cells, other than is above described, been seen. This cellular layer is bounded by a fine basement membrane separating it from the underlying basis of the villus; and this basis consists of a column of

connective tissue fibres and cells, generally somewhat loose in texture, and containing a large thin-walled blood vessel. This connective tissue basis contains a very variable proportion of cellular material; in some places there is a marked small-cell infiltration, whilst for the most part this character is not at all well marked. The villi so formed vary much in size, the larger, which by branching produce the smaller, only differing in that they contain more connective tissue. As already mentioned, the deeper parts of the tumour are more solid than the anterior, the villi being larger and the cystic space much less. Finally, the deepest part of all is almost solid, and consists of tissues similar to and continuous with those of the central columns of the villi. In no section cut has a sign of a capsule to the tumour been seen.

It is clear from the above description that the tumour is characterised by an immense proliferation of the epithelial constituents of the thyroid gland, far outstripping in interest the accompanying increase in stroma. In fact, as Professor Delépine pointed out to me, the histological features are very similar to those of the proliferous or papillary cysts found in connection with the hilum of the ovary, and to the intracystic papillary growths present in the dilated mammary ducts in several forms of tumours.

Taking the primary growth alone into consideration, there would be no good reason for coming to any conclusion as to its being other than benign. As there is in no part a carcinomatous structure, there need be no expectation of finding secondary deposits. It is, however, almost certain that such deposits have occurred. On cutting sections of the smaller appendages of the tumour already mentioned as having been removed along with it, in each case exactly the same appearances are seen as in the larger growth. The only difference to be observed here is that the epithelial cysts containing the papillary growths are surrounded by a zone clearly consisting of lymphoid tissue; this in turn is surrounded by a distinct capsule. There seems at first sight no reason to doubt that these structures are lymphatic glands containing secondary malignant deposits. On further consideration, however, the problem is not so simple, for accessory thyroid glands may occur in the situations in which these were found, and such accessory glands may on certain occasions take on the characters of a thyroid, itself the seat of disease. Such accessory thyroid glands are of common occurrence, and present, as the thyroid itself does, a certain, though indefinite amount of lymphoid tissue. Though these considerations take away any absolute certainty as to the presence or absence of secondary malignant deposits, it seems in the highest degree probable that the smaller growths are in reality of such a nature. In favour of this conclusion the following points may be noted:—

1. The three smaller growths all occurred on the same side as the primary one, and lay in the course of the normal lymphatic current

from the right lobe of the thyroid to the thorax. If they were merely sympathetically enlarged accessory glandules, it does not seem probable that they would have had a purely unilateral distribution so closely aping that of the usual lymphatic glands.

2. The structure of these bodies exactly resembles that of lymph glands in which secondary deposit has taken place. In the primary tumour there are a few patches resembling lymphoid tissue, but no sign of a large infiltration. It seems, therefore, that the layer of tissue in which the smaller deposits lie acts, not as a constituent of the mass of which it forms part, but rather as a sort of host to a secondary parasitic malignant growth.

3. In Pollard's case, what were apparently enlarged lymphatic glands were found in the supraclavicular region at the same side of the tumour; unfortunately these were not examined.

Professor Delépine, who examined a similar case some years ago, is of opinion that the tumour referred to in this paper is a malignant adenoma or epithelioma (according to the nomenclature used). If it were not for the evidence, which tends to show that in this case there were secondary growths in the cervical lymphatics, he would have called the tumour a "papilliferous cystadenoma." The resemblances which exist between this neoplasm of the thyroid body and papilliferous cystic tumours of the ovary and of the mamma, does not preclude the idea of malignancy, for Dr. Delépine has seen apparently typical proligerous papillary cysts of the ovary give rise to metastatic growths of exactly the same structure, in the abdominal lymphatic ganglia. Cornil, in his excellent paper on a similar tumour (*loc. cit.*) in 1875, came to the conclusion that this form of growth of the thyroid body must be placed among the epitheliomata, and that it shows great affinity with the cylindrical epithelioma from which, however, it differs. The plate accompanying Cornil's paper leaves no doubt as to the close resemblance between the tumour described by him and the one forming the subject of this paper.

ON POST-MORTEM NERVE CHANGES.

By S. RUSSELL WELLS, M.B., B.Sc., M.R.C.P. (Lond.), *Assistant Curator of the Pathological Museum, St. George's Hospital, London*; and
W. H. WILSON, M.A., M.B. (Oxon.), *late Radcliffe Travelling Fellow, Oxford.*

From the Pathological Laboratory, St. George's Hospital, London.

(PLATE XXIII.)

HISTORICAL.

SEVERAL observers have described in neuritis changes in the nerve fibres themselves, namely, certain alterations of the medullated sheath, by which it first becomes in places finely granular, then loses its power of being stained by osmic acid, collapse of the nucleated sheath, and in some cases changes in the axis cylinder which lead to its rupture, this rupture being followed in the part of the nerve below by the phenomena of Wallerian degeneration, which are so well known that description is needless. It may be well to refer briefly to some of the more important papers in which such conditions are described. Sigmund Mayer¹ teased the nerves of winter frogs and normal rats in $\frac{1}{2}$ per cent. salt solution, and stained with osmic acid, counter-staining with fuchsine or picrocarmine. He found in some fibres the following appearances:—(1) Medulla broken into pieces of various sizes, lying between which could be seen finely granular matter; (2) medulla broken into fine granules in portions of the nerve; (3) medulla unstained by the osmic acid in places, the nerve appearing as if it consisted of only the nucleated sheath and axis cylinder, with here and there a few unstained granules between them. He considers the changes may be reduced to two phenomena, *i.e.*—

1. Breaking up of the medulla into granules,

2. Absorption and disappearance of these granules;

and that there is no apparent difference between these "normal" degenerative changes, which occur in a few fibres of a nerve, and those seen in a nerve after section.

¹ *Sitzungb. d. k. Akad. d. Wissensch.*, Wien, 1878, bd. lxxvii. Abtheil. iii. s. 80.

Gombault¹ subjected guinea-pigs to chronic lead poisoning by giving them white lead. No paralysis was produced, they were killed some months after treatment. The nerves were teased and stained in osmic acid and counter-stained with carmine, when it was found that many nerves showed at irregular intervals, between healthy portions, granular breaking down of the medullary sheath. Others had progressed further to a disappearance of this granular matter (want of staining by osmic) and collapse of the primitive sheath, in places, while in yet other nerves nothing was seen of the medulla except scattered collections of granules and nuclei. Gombault says that these changes may go on to either rupture of the axis cylinder and Wallerian degeneration, or regeneration may take place. He sums up the changes in lead neuritis as follows:—

1. Granular breaking down of the medullary sheath, starting at the nodes, and progressing towards the internodal portion. This lesion is discontinuous in the same fibre, and attacks some fibres to the exclusion of others.

2. The axis cylinder is not, as a rule, affected.

3. The myeline sheath and protoplasm are only affected.

Martin² describes similar changes in the nerves of animals poisoned by injection of the chemical products produced by the activity of the diphtheria bacillus. Sometimes a small nerve was affected right across at several points, becoming attenuated, and unstained by osmic acid, in others degenerated fibres could be found between healthy ones. His description is very well summed up in the following quotation:—

“It is clear . . . that the condition is one of simple degeneration; affecting, first of all, the white substance of Schwann, which breaks up and finally disappears, and affecting later the axis cylinder, which becomes attenuated and finally ruptured in some fibres, so that below this ruptured axis cylinder the fibre undergoes the Wallerian degeneration.”

The same author³ describes similar appearances in the nerves of 2 patients who had died of tubercle, and had been of alcoholic habits. In this last instance the nerves were taken at the post-mortem examination, 43 and 44 hours after death; while, as all the previously mentioned observations were made on the nerves of animals, we may assume that the nerves were taken as soon as possible after death.

METHODS ADOPTED IN THIS INVESTIGATION.

The present writers determined to study the microscopical changes which take place in the nerves after death, with a view to seeing if

¹ *Arch. de neurol.*, Paris, 1880-81, vol. i. p. 11.

² *Rep. Med. Off. Privy Council*, London, 1891-92, p. 147.

³ *Journ. Path. and Bacteriol.*, Edin. and London, vol. i. p. 322.

changes at all comparable to those described above could be detected. Portions of the vagus nerve, near the point where the recurrent laryngeal nerve comes off, and of the nerve to the tibialis anticus muscle, near its point of entrance into the muscle, were taken at the post-mortem examination, from the bodies of 6 patients, and immediately put into 1 per cent. osmic acid solution. Other pieces of the same bit of nerve were wrapped in a piece of muscle and left to decompose at the temperature of the laboratory, and after intervals of time, varying up to 159 hours after death, parts of each were taken and placed in 1 per cent. osmic acid. When these nerves had been in the osmic acid for 24 hours, the perineurium was teased off and they were replaced in the acid and left in it for several days, in order to make certain that the whole nerve was thoroughly impregnated with the solution, and all parts that would take the dye perfectly stained. The nerves were next rapidly washed in water, and in some cases teased at once, others were left in methylated spirits until convenient to treat further. As a rule, the nerves were teased in and mounted in Farrant's medium, in order to avoid, as far as possible, the fear of altering their appearance, since it seemed to us very likely that the fatty substance might be dissolved out in the processes of dehydrating in absolute alcohol, clearing in oil of cloves, and mounting in xylol balsam. A few, however, were mounted in balsam, and we may here remark that though we consider Farrant to be the more suitable medium, yet mounting in balsam does not seem to very materially alter the appearances. Some of the preparations were counter-stained with borax carmine, which is particularly suitable for bringing out the axis cylinder and nuclei. Altogether a very large number of preparations was made, representing each nerve at very varying intervals after death. None of the 6 patients, from whose bodies the nerves were taken, showed any symptoms during life that could be referred to peripheral neuritis, or like condition. One of them, it is true, was a man, æt. 29, who had a malignant tumour of the cerebrum, but he died of broncho-pneumonia, and his nerves presented exactly the same conditions as those found in the other cases now to be enumerated.

F. P., male; æt. 28.—Empyema and pericarditis.

C. P., male; æt. 43.—Phthisis.

W. H., male; æt. 34.—Vegetative endocarditis.

J. H., male; æt. 22.—General peritonitis, following perforated appendix.

G. G., male; æt. 2½.—Broncho-pneumonia.

It will be observed that most of the cases were of an acute nature.

APPEARANCES FOUND.

The appearances found varied markedly according to the length of time which had elapsed between death and the fixation of the nerve in osmic acid. The earliest change found, which we have seen as late as

the sixteenth hour after death, is simple breaking of the medullary sheath transversely; at this stage the nerve fibres stain well, and neither areas of partial staining nor absence nor granulation of the medulla can be detected. Next, the medulla becomes broken up into granules, some fibres appearing to be slightly swollen. Concurrently with this, deficient staining power affects many of the fibres, which, however, may lie between others that have taken the stain well; or this want of staining may be confined to a small length of the fibre, to be followed by a normal portion, which may again be interrupted by an unstained area, so that in one and the same nerve fibre several stained and unstained portions may alternate. Probably the want of staining is due to loss of the myeline, for the unstained parts are generally constricted. In other nerves, and this we take to be a later stage, the want of staining and loss of medulla may extend across the whole thickness of a nerve bundle at intervals; where this occurs the nerve appears to be thin and bends readily, often being somewhat kinked or twisted. This, at one time, nearly led us to suppose that the nerves presented the changes above described as the result of mechanical injuries; we do not, however, now think that this is the case, but consider that the loss of medulla is due to chemical post-mortem changes, and that the consequent softening leads to the bending at these points. When the above changes in the myeline are markedly present it is impossible to find the nodes of Ranvier; they seem to be destroyed by the process of disintegration which the medullary sheath undergoes.

The fibres in which absence of staining and loss of medulla is not seen at this stage may appear to possess a moniliform outline, with aggregation of myeline at some points along the fibre, in others partial absence, together with the granular disintegration mentioned above, giving it a very irregular appearance. The last stage of all observed was seen in nerves that had been placed in osmic acid from 100 to 150 hours after death; here the appearances of partial staining and localised loss of medulla are merged in the general and extreme changes that the medulla undergoes. The nerve tubes are, in some places, filled with fatty globules stained black, which often distend the primitive sheath; a fine granular débris may be seen in many, some tubes appear quite empty, while others contain a few scattered drops of myeline.

The axis cylinder appears generally not to take part in the above changes, as, if stained with borax carmine, it can, as a rule, be seen intact, even in the advanced stages; but occasionally we have seen it ruptured, and in some cases stained black with osmic acid. The primitive sheath also, though it may collapse, as far as we have been able to observe, remains unaltered. The loss of the medullated sheath produces such a profound change in the appearance of the nerve, in consequence of the loss of osmic staining and collapse of the nucleated

sheath, that in the later preparations it is very hard to recognise the nerve fibres as such, and they come to resemble connective tissue fibres. With regard to the length of time necessary for the development of the different stages, it is impossible to give any exact numbers, as the changes are run through more quickly in some nerves than others, and, doubtless, various factors are concerned, such as the cause of death, the temperature, weather, etc. It is important, however, to note that segmentary staining and fine granulation have been observed as early as 20 hours after death.

COMPARISON OF POST-MORTEM CHANGES WITH THOSE DESCRIBED IN NEURITIS.

Comparing these changes with those described in neuritis, it will be seen how close is the resemblance; indeed, most of the figures given by the authors referred to at the commencement of this paper might be taken as representations of our specimens. The segmentary staining on which so much stress has been laid, and which is certainly not due to want of penetration of the osmic acid, is remarkably well shown in many of our slides, as is also the granular breaking up of the medullary sheath. We cannot say that we have so frequently seen rupture of axis cylinder, as the writers on neuritis seem to have done, and, of course, any real proliferation of the nuclei does not occur, any more than regenerative changes. The later changes bear a certain resemblance to Wallerian degeneration. In illustration of the similarity to the nerve changes figured in neuritis, we may mention that on showing our slides to a gentleman of much practical experience in pathology, the inquiry was made as to whether the specimens were from cases of diphtheritic or alcoholic neuritis. The question now arises, Were all the appearances, which have been described as the results of neuritis, due to post-mortem alteration? In the majority of cases this could not have been so, as the nerves were obtained from animals, presumably immediately after death.

CAUSE OF THE CHANGES.

It is well known that nervous tissue is one of the most unstable parts of the body; for instance, that brain may be softened when the muscle from the same animal is perfectly sound. It seems to us very probable that its decomposition always proceeds along similar lines, so that whether the nerve dies, as in Mayer's observations, in the body, as the result of a natural process, or is poisoned by lead, alcohol, or diphtheritic poisons, or dies as the result of systemic death, the microscopical changes it goes through on the road to disintegration will be always the same. Analogies are proverbially apt to be misleading, but as an illustration of our meaning may be instanced

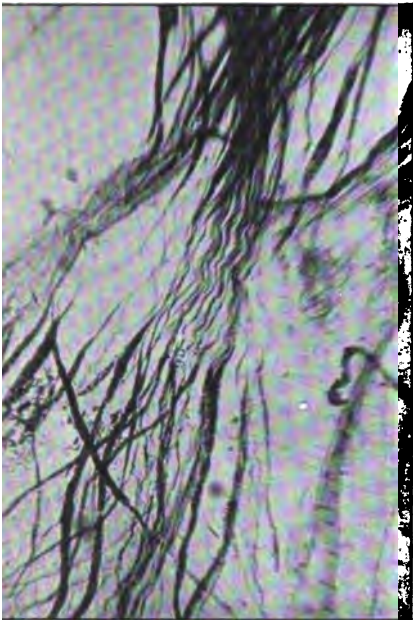


FIG. 2.

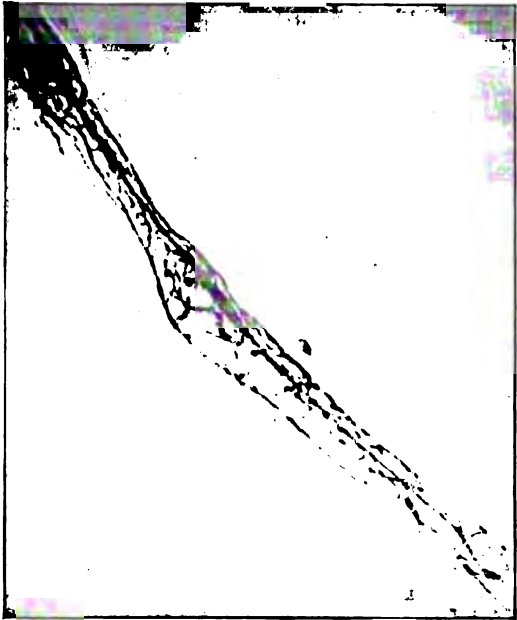


FIG. 4.



FIG. 1.

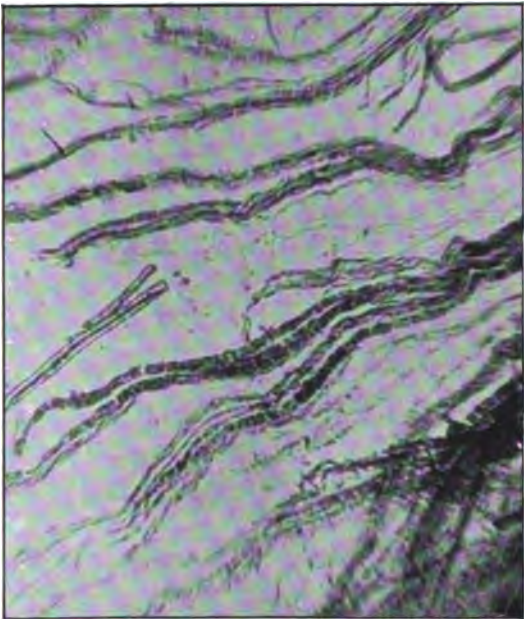


FIG. 3.

1. The first part of the document is a list of names and dates.

2. The second part of the document is a list of names and dates.

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coagulation of the blood, which may take place either inside or outside the body, as the result of apparently very varied stimuli, yet always runs through the same naked-eye and microscopical changes.

CONCLUSION.

In conclusion, we would most emphatically protest against any similar alterations found in human nerves, as usually obtained post-mortem, being taken as evidence of neuritic change, for it would be almost impossible to prove that they were not of post-mortem origin. We wish to take this opportunity of thanking Dr. Rolleston for the facilities he has afforded us for making this investigation.

DESCRIPTION OF PLATE XXIII.

- FIG. 1.—Nerve bundle showing a segment in which the medulla is completely lost in many of the nerve fibres.
- FIG. 2.—Bundle of nerve fibres showing a segment in which medullary sheath is almost entirely absent.
- FIG. 3.—Granular disintegration of the medulla, the result of post-mortem change.
- FIG. 4.—Nerve fibre in an advanced state of post-mortem change. Swollen medullary sheath in great part absent. Axis cylinder stained with osmic acid, swollen in places.

OBSERVATIONS AND EXPERIMENTS ON THE PATHOLOGY OF GRAVES'S DISEASE.

By WALTER EDMUNDS, M.A., F.R.C.S.

(PLATES XXIV. TO XXXIV.)

MICROSCOPIC examination of the enlarged thyroid constituting an ordinary goitre shows various changes; there are found—

1. A tissue differing from the normal thyroid only in being somewhat coarser.
2. Cysts, some containing colloid material, and some a papillomatous ingrowth.
3. Nævoid or erectile tissue: this explains the expansile pulsation and thrill felt in some goitres, and also the hæmorrhage which during operations sometimes occurs from the thyroid tissue itself: this as a rule does not bleed, any hæmorrhage which arises coming from the capsule, which is highly vascular (Plate XXV. Fig. 9).
4. Myxomatous changes of the interacinous tissue; this tissue then stains of a pale colour, and thus contrasts with the colloid contents of the vesicles. Sometimes the vesicles contain in their centre normal dark-staining colloid, and external to this, next the lining cells, a layer which stains much more palely (Plate XXV. Fig. 6 and Plate XXIV. Fig. 34).
5. Tissue of an embryonic type, consisting mainly of secreting cells, and not containing either vesicles or colloid (Plate XXIV. Fig. 5 and Plate XXV. Fig. 6).

The goitre of Graves's disease does not differ greatly from others, but a remarkable hypertrophy of the blood vessels is sometimes found, and the presence of the "embryonic" (small-celled) tissue is, as Greenfield points out, fairly constant (Plate XXXII. Fig. 30 and Plate XXIV. Fig. 5).

This tissue resembles that of the parathyroid glands.

These glands are recognised most easily in rabbits, for in them they are situate quite apart from the thyroid proper; they are of a bright red colour, and lie one on each side of the trachea below and at a considerable distance from the thyroid. They consist mainly of secreting cells arranged more or less in columns; there are no vesicles

and no colloid; these glands were first described by Sandström in 1880 (Plate XXVIII. Fig. 20 and Plate XXX. Fig. 26).

In rabbits it has long been noticed that excision of the thyroid gland is not followed by the same fatal result that attends the operation almost (though not quite) invariably in dogs and cats; but Gley has recently shown that in rabbits, if, as well as the thyroid, the parathyroid glands are removed, the animal, as a rule, dies. These experiments of Gley's have been repeated by myself, and it was found (1) that if both the thyroid and the parathyroid glands are removed the animals die; out of a batch of 7 rabbits, on whom this operation was performed, 5 died within 8 days, the other 2 surviving for months. (2) That when the thyroid gland only is excised, the parathyroid glands being left, many of the animals also die; in a batch of 7 operated on, 5 died within 42 days, and 2 survived for months; in another set of 17 operations, 2 were killed while in good health (at the fifty-second and the fifty-ninth day), and the remaining 15 all died within 97 days; in 4 of them there was noticed a condition resembling myxœdema in man; the general health failed, the hair fell out, and there was a remarkable œdema of the lower part of the face. (3) If the parathyroids are alone extirpated, the animals, as a rule, live, and do not undergo any obvious change, at least not for a long time. In a batch of 7, one died at the thirty-fifth day, a second the eighty-second day, and a third the one hundred and fifty-sixth day; the other 4 survived; 2 of these now, after 6 months, are weak and emaciated.

The appearance of some of these parathyroidless rabbits gave rise to the suspicion that the operation caused exophthalmos, and a fresh series of experiments (with controls) was made to test this point; the result seemed clear, the operation did not cause exophthalmos,—indeed, after the lapse of 6 months, the eyes in 2 or 3 of these operated rabbits looked sunken, as if they were suffering from the opposite condition of exophthalmos. It is therefore proposed to make another series of experiments to decide as to this.

The parathyroids after extirpation of the thyroids do not undergo any marked changes, but they may hypertrophy somewhat; under the microscope, too, there is no pronounced alteration of minute structure; they do not develop into normal thyroids; no vesicles, and no colloid form (Plate XXVIII. Fig. 19).

In dogs, also, there are parathyroids, but in them these small glands lie half-embedded in the substance of the thyroid itself; consequently, in excision of the thyroid lobes, the parathyroids have been removed as well (Plate XXVIII. Fig. 16). Gley found that if the parathyroids are separated and left, the animal will live, notwithstanding the removal of the rest of the thyroid lobes. The present writer's experiments (6 in number) show that if the whole of one lobe of the thyroid, including its parathyroid, and also the

greater part (two-thirds or more) of the other lobe be removed, the animal will live or die, according as the parathyroid is or is not left.

The parathyroid, and also the portion of thyroid that is left in these experiments, hypertrophy considerably.

The hypertrophied parathyroid consists only of new columns of secreting cells, and has not developed into thyroid proper; no vesicles nor colloid formed in the two specimens I examined.

The hypertrophied portion of thyroid proper contains a considerable growth of new tubules lined with a single layer of secreting cells; there also appears to be less colloid in the vesicles, as if it had been absorbed; in one case many vesicles were empty (Plate XXVIII. Fig. 18 and Plate XXIX. Fig. 21). Further, the cells lining the vesicles are greatly hypertrophied, as described by Hürthle; they have become much larger than natural, and generally both in normal and enlarged thyroids the larger cells seem the more active cells, and this throws some light on the "vacuoles," which are found in both normal and diseased thyroids in the periphery of the colloid in the vesicles. The so-called "vacuoles" are to be seen in alcohol-hardened specimens; and, from the fact that they are not present after osmium fixing, it has been supposed they are due to shrinking from the action of the alcohol, but they seem too regularly disposed for this, and their absence from the vicinity of flat (non-acting) cells points to their probably consisting of tiny portions of recent secretion, which has not yet become mixed with the rest of the colloid.

Later experiments show that a dog can live with only one parathyroid, that is to say, after the other parathyroid and the whole of the thyroid proper have been removed. If both parathyroids are removed, the dog appears to require at least three-quarters of one of the two thyroid lobes.

Parathyroids also occur in the sheep, monkey, and in the human subject. Creswell Baber has found them in the seal (Plate XXV. Figs. 7 and 8, Plate XXVI. Fig. 1, and Plate XXIX. Figs. 22 and 23).

The parathyroid gland is, it is clear, of considerable importance; it is not quite so important as the thyroid gland, but, bulk for bulk, it probably is more so, as it is considerably the smaller. In a rabbit weighing 1800 grms. the thyroid weighed 220 mgrms., and the parathyroids together 19 mgrms., or about one-twelfth of the thyroid.

Although the tissue of the parathyroid gland does not at all resemble that of the thyroid in its adult form, there can be little doubt that they are closely connected, not only on account of one being able, to a great extent, to replace the other physiologically, but also because—(1) the parathyroid resembles the embryonic form of the thyroid; (2) the two tissues are occasionally found side by side in the parathyroid of the dog; and (3) because they are closely connected anatomically; in the monkey the parathyroid is embedded in the substance of the thyroid.

Now, it has been supposed that the symptoms of Graves's disease (other than the goitre) are due to the action of the internal secretion from the enlarged thyroid; there, however, exists little, if any, evidence that thyroid secretion can produce exophthalmos. The eating of the thyroid of sheep, or the receiving of the subcutaneous injection of the extract, has produced unpleasant symptoms in healthy persons; in the subjects of myxœdema an excessive dose has produced grave and, it is stated, even fatal symptoms, but it has seldom, if ever, appeared that the eye symptoms of Graves's disease were among these effects.

In animals, thyroid feeding produced, in my hands, no very obvious results, certainly no exophthalmos. To a healthy dog were given, in 1 day, the thyroids of 16 sheep, without any apparent result, and to another dog were given sheep's thyroids, 2 per diem, for some days without effect. Also monkeys were treated daily with large doses of the extract subcutaneously, without the production of obvious symptoms, except that in one case, about the time of stopping the treatment, suddenly an area of baldness appeared on each temple, extending downwards to the shoulder; this hair gradually grew again, but an attempt to reproduce the result in the same monkey by the reapplication of the supposed cause, *i.e.* the administration of thyroid extract in large doses and then suddenly stopping it, failed.

If it be argued that the thyroid of Graves's disease is not merely an enlarged one, but is also altered in structure, and that therefore the secretion is also probably altered, it must be answered that that may well be, but that no evidence has yet been brought forward that this altered secretion can produce exophthalmos. It would certainly be desirable, as has been suggested, to try, when opportunity offers, the physiological effect of the secretion found in the enlarged thyroid in Graves's disease.

The apparent contrast between the symptoms of Graves's disease and myxœdema, coupled with the brilliant success which has attended the treatment of the latter, and also of cretinism¹ by thyroid taking, certainly helps the secretion theory. But there are one or two considerations that go against it.

1. The contrast between Graves's disease and myxœdema only holds good with chronic myxœdema; in the acute myxœdema as seen in dogs, and sometimes in monkeys, there are tremors, with attacks of dyspnoea resembling those of Graves's disease.

2. Two cases have been described (by Sollier) of the coexistence in the same patient at the same time of Graves's disease and myxœdema. Zum Busch has recently recorded a case of Graves's disease in which myxœdema supervened and partially replaced the symptoms of Graves's disease; the myxœdema was cured by thyroid treatment, but exophthalmos and Graves's sign remained.

¹ Plate XXVIII. Fig. 17.

3. Thyroid feeding does not as a rule make cases of Graves's disease worse. Dr. Mackenzie tells me he tried the treatment in a series of cases without marked result in either direction. Auld has, however, recorded a case in which injurious results followed, and appeared to have been caused by the treatment. I have heard, too, of one or two other cases in which the same effect has been observed; but this effect is, I submit, the exception, and not the rule.

4. The difficulty of saving by thyroid treatment animals deprived of their thyroids tells against the secretion theory. Twenty dogs, whose thyroids were excised, were treated by thyroid feeding, or by the administration of the extract of thyroid: the details of the treatment were varied, and also of the operation; the thyroid in some cases was removed in stages. The total result was that only 2 out of the 20 were saved: this is, however, more than could have been expected without treatment, judging from the experience both of myself and others. The survivals are stated to be less than 5 per cent.; moreover, the dogs lived a few days longer than they would have done without the treatment, and the symptoms were much modified, for the acute attacks of dyspnoea and rapid breathing were absent, the animals dying of emaciation and asthenia.

It is worthy of note that in neither of the two cases saved were there any symptoms, and that after the lapse of a few weeks the treatment was entirely stopped, without ill effects. In one of the cases a post-mortem examination was made, and no trace of thyroid tissue was discoverable. The pituitary body was larger than that of another dog of about the same size, but this I am disposed to regard as merely accidental.

In monkeys, 8 were treated with thyroid extract administered subcutaneously in all cases but one; they all died in from 12 to 128 days, the average time of survival being 44 days. The symptoms from which they suffered were those of myxœdema in monkeys, as described by Horsley; they lost weight, became less lively, respiration became slower, hair fell out in places, œdema appeared in the face, and the pupil was somewhat dilated; tremors occurred, and sometimes convulsive attacks, with rigidity of limbs.

Stanley Kent's results in cats are in accord: of 5 submitted to thyroidectomy and treated he saved only 1; and of 4 in which the thyroid, and also one or both testes, were removed, and the treatment followed, 2 died, 1 was killed while ill, and only 1 survived.

Another argument for the secretion theory is found in the cure or amelioration of Graves's disease, which, now, many times has followed the removal of the whole or part of the enlarged thyroid. One of the latest to review these cases is Dr. Oppenheimer, of Baltimore, who finds a total of 68 on record: of these, 18 are said to have completely recovered; 26 were more or less improved; in 9

there was no change either way; in 5 there was immediate death; and in 4 death followed the operation within 24 hours.

One case of improvement, amounting practically to cure, has come under my own observation. The case was seen almost from the commencement of the symptoms, and treated, but without success; it gradually became worse, the patient at last being very ill, with the usual symptoms, including the paroxysmal attacks of palpitation and rapid breathing. The only course that seemed left was to operate on the goitre, but before undertaking this step a consultation was held with Dr. Hector Mackenzie. He agreed in the advisability of the operation, and also in considering the spasmodic attacks to be due to the effects of the disease itself, and not to the mechanical pressure of the goitre on the trachea. The attacks resembled those which occur in thyroidless dogs. A considerable portion (but not all) of the goitre was removed; the patient was much benefited, and the symptoms gradually passed off, so that the patient was practically well, though the pulse, if counted, was found too fast, a little exophthalmos might still have been detected on critical examination (at no time was it a prominent symptom), and occasionally an attack of dyspnoea occurred.

It is argued that the improvement must be due to the diminution of the thyroid secretion, following the removal of a portion of the thyroid, and that therefore the symptoms are due to that cause; but it must be remembered that for these cures to take place it is not apparently necessary that the whole or nearly the whole of the goitre should be removed; in some cases only one lobe has been excised, the remainder atrophying in time. This, too, is only what occurs in operations on ordinary goitre. In a case in which about half of a considerable goitre (which was compressing the trachea) was removed, the remaining portion gradually atrophied, so that in about a year the thyroid could not be detected, and the patient was well. These latter cases can only be explained by the breaking of some vicious circle, and the same explanation may apply in the cases of Graves's disease. Moreover the improvement, as we have seen, does not always follow: Dr. Mackenzie informs me of a case under his care of 10 years' standing, in which a considerable portion of the goitre was excised, in the hope of benefiting the exophthalmos, which was extreme; the patient was not improved, certainly not in the exophthalmos.

The fact that the eye symptoms of Graves's disease can be produced by a chemical poison (cocain) may be held to support the view that a poison secreted by the thyroid might do the same.

The effects of cocain in this connection were first pointed out by Koller, and have since been carefully studied by Jessop; the latter found that by dropping cocain into the eye there was produced—(1) proptosis, (2) absence of winking, (3) Graefe's sign, (4) local anæsthesia, (5) dilatation of pupil, (6) widening of the palpebral

fissure, (7) paralysis of accommodation, (8) diminution of ocular tension.

In a case of complete facial paralysis cocain still caused widening of the palpebral fissure, and he argues that the cocain must act on the unstriated muscular fibres (which are supplied by the sympathetic). His experiments also show that stimulation of the sympathetic in the neck could further dilate a pupil already as fully dilated as atropine could make it; and that cocain could also dilate a pupil fully dilated with atropine. In a case of Graves's disease he found that cocain administered cautiously produced increased proptosis, further dilatation of palpebral fissure, and halting in the descent of the upper eyelid.

Jessop also found that in the rabbit, if the cervical sympathetic be divided, after a few days cocain will not produce dilatation of pupil, nor proptosis, nor widening of the palpebral fissure.

The effects of cocain injected subcutaneously in monkeys, both with and without division of the cervical sympathetic, have been tried by myself. The results in monkeys of division and stimulation of the cervical sympathetic are described by Sherrington in the *Journal of Physiology*, and represented in the figure here reproduced.



FIG. 1.—Shows effects on division of right cervical sympathetic nerve.
Sketch *ad nat.*

Division of cervical sympathetic causes—(1) recession of eyeball, (2) contraction of pupil, (3) narrowing of palpebral fissure, (4) œdema and flushing of skin round eye, (5) swelling of caruncle, (6) projection of pinna from side of head, (7) puckering of skin of muzzle, (8) flattening of certain hairs on forehead, which cannot then

be elevated by the emotions which will raise the corresponding hairs on the opposite (normal) side of the head.

Stimulation of the cervical sympathetic, on the other hand, produces—(1) proptosis of the eyeball, (2) dilatation (well marked) of pupil, (3) widening of palpebral fissure, (6) lying back of pinna, (8) erection of certain hairs on forehead.

The solution of cocain used was the hydrochlorate, and it was found that 2 grs. of the salt ($= 0.13$ grm.) was fatal to a monkey. Immediately after the injection he jumped about in an excited manner; then his movements became less precise, and soon he had to hold on to the side of his cage to retain the erect attitude; then his hold relaxed, and he collapsed on the floor of the cage, and had a succession of attacks of clonic spasms, during which the arms were extended and the hands clenched, the head partly thrown back, and the upper eyelids retracted. These attacks lasted about 4 seconds, with intervals of 15 seconds. Gradually the respiration became feebler, and notwithstanding artificial respiration he died in 20 minutes or half an hour from the time of injection. After death it was noticed that the eyelids were unusually widely open, the upper eyelid being retracted, and that the eyes were prominent (as shown by comparison with a normal monkey). Half an hour after death the cornea was still clear and convex instead of hazy and flaccid, as it usually becomes.

The cocain experiments were made on 12 monkeys altogether. The drug was injected subcutaneously in half-grain doses ($= 0.032$ grm.) once a day, but it being found that this when continued produced death, the dose in the later experiments was reduced to a third or a quarter of a grain. Five of the monkeys died certainly from the effects of the injections, and 3 others probably from the same cause; 2 of the 5 died in convulsions.

The effect of the injections caused the animals to seem dull and to lie down,—indeed, they appeared unable to stand. Sometimes there ensued an attack of convulsions; these effects passed off in about an hour, and there remained exophthalmos, dilatation of the pupil, and widening of the palpebral fissure, and (as was thought) increased intraocular tension.

In three cases thyroid extract was injected as well as cocain, but its addition made no difference that could be detected. When the cocain injections were stopped the symptoms at once ceased: it seemed impossible to start a disease in any way resembling exophthalmic goitre by administration of cocain or thyroid extract, either separately or together.

In 7 of the monkeys, a few days before commencing the cocain injections, a long piece of the sympathetic nerve in the neck was excised. The effects of the excision were to produce contraction of the pupil (which came on as the effects of the anæsthetic passed off).

and retraction of the eye ; the effect of cocain subcutaneously on such a monkey is to greatly dilate the pupil on the normal side, and to cause proptosis there. On the operated side the pupil is somewhat dilated, but not so much ; apparently no proptosis is produced, but it is not easy to be absolutely certain of this, as the opposite side is no longer normal for comparison ; if there is any proptosis it certainly is not much, for the eye is not nearly so prominent as on the unoperated side. This effect of division of the sympathetic is very important, for it affords an indication for treatment in these cases of Graves's disease, in which the prominence of the eye is so great as to cause ulceration of the cornea. The usual treatment is to partially or wholly close the palpebral opening by suturing the eyelids together, but this is not always successful ; sometimes, notwithstanding this, and also strapping the lids together, the cornea sloughs, and the eye (or even both eyes) is lost. The effects of paralysis of the sympathetic in man are not serious—contraction of pupil and absence of perspiration on that side ; the affection is generally only discovered accidentally.

The effects of cocain, then, it must be admitted, show the possibility of a poison being secreted by the thyroid which might cause symptoms like those of Graves's disease.

The real issue is whether the goitre of Graves's disease is primary, and by its secretion the cause of the other symptoms, or whether the disease is primarily of nervous origin.

In favour of the latter view it may be said that the relations of the disease are not so much thyroïdal as neurotic.

The majority of those who have had an opportunity of judging appear to think that Graves's disease is not more common in goitrous districts than elsewhere. On the other hand, it has many nervous connections. Solbrig relates the case of a boy aged 8 (the son of a woman who suffered from the disease), who, after a disappointment at school, was seized with palpitation and profuse sweating ; the next day the thyroid was large, the eyes prominent, and the pulse 180. Two days later the symptoms gradually disappeared, and in 10 days he was well again. Putnam cites from Coggeshall an exactly parallel case. The patient was a young girl, and the symptoms followed immediately on great excitement attending a whipping, but subsided in a few days.

Again, certain experimental lesions of the central nervous system (of the restiform bodies) have been stated by Filehne and Bienfait to be capable of causing the symptoms of Graves's disease, namely, tachycardia, exophthalmos, and hyperæmia of the thyroid (but not a definite goitre). Further, Mendel found post-mortem, in a case of Graves's disease, atrophy of one restiform body.

The disease, too, has some relations with diabetes. Dr. Acland had recently under his care in St. Thomas's Hospital a patient suffering from diabetes, and passing between 4 and 5 oz. of sugar in the

24 hours. Eighteen months previously the patient had been admitted with Graves's disease, suffering from a considerably enlarged thyroid and palpitation; on his second admission the thyroid was much smaller, though still enlarged, and the palpitation absent though the pulse was still quick.

An irritation of the sympathetic nerve, either applied to its origins in the central nervous system (brain or cord) or to its prevertebral ganglia, would readily enough account for both the cardiac and the ocular symptoms; as to the hypertrophy of the thyroid, it has been shown that stimulation of the sympathetic causes an increased secretion of the solid constituents of the saliva; the thyroid closely resembles the salivary glands, differing from them mainly in not draining through a duct, and it may be conjectured that prolonged stimulation through the sympathetic might cause the hypertrophic changes. The thyroid contains similar granules to those found in the secreting cells of the parotid gland (Plate XXX. Fig. 25).

Again, occasionally the eye symptoms are unilateral, and then the hypertrophy of the thyroid is generally greater on the same side as the affected eye. It was so in a case recorded by Maher, of Sydney.

The great improvement, if not recovery, in which Graves's disease in many cases terminates seems to negative any pronounced central lesion, and we need not therefore be surprised that none has been with certainty yet established.

Somewhat against the secretion theory is the fact that no poison has yet been found in the blood or spleen in experimental athyroidia. The blood from a dog dying of acute myxœdema, following thyroidectomy, was drawn and defibrinated, and injected into a vein of a normal dog: no effect—certainly no permanent effect—was produced. This experiment was tried five times. Also the albumoses was extracted from the spleens of dogs dead of athyroidia. Mr. White, pharmacist to St. Thomas's Hospital, kindly did this for me. The principle of the method depends on the facts that alcohol precipitates in the spleen the albumoses, the albumens, and the globuloses. At the end of about 3 months the last two are insoluble in water, while the albumoses are still thus soluble. They are dissolved, and the bulk is concentrated by evaporation at a low temperature and barometric pressure, and precipitated again by alcohol, and this is repeated several times in order to obtain a pure product: the albumoses thus obtained were injected subcutaneously into guinea-pigs with an entirely negative result. It would probably have been better if the injection had been intravenous.

The experiments related above were made at the Brown Institution, and the writer has much pleasure in expressing his thanks for the opportunities afforded him.

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DESCRIPTION OF PLATES XXIV. to XXXIV.

PLATE XXIV.

FIGS. 1, 2, 3 and 4. Sections from the goitre in a case of Graves's disease.

A portion of the goitre was removed by operation, and from this the sections made. There will be noticed (1) the large amount of young secreting (thyroid) tissue between the vesicles; (2) the hypertrophy of the secreting cells lining the vesicles; (3) the multiplication of these cells; in some vesicles they completely fill the cavity; (4) in Fig. 4 an inflamed artery and also a vein containing blood. ($\times 380$.)

These and all the other specimens were obtained as fresh as possible, fixed in Foa's solution, and hardened in alcoholic solutions of gradually increasing strength; they were embedded in paraffin. Various staining reagents were used, the most satisfactory being logwood and eosin, logwood and rubin, methylene-blue and rubin, and the Ehrlich-Biondi-Heidenhain stain. The drawings were made by Mr. Lapidge with Powell and Lealands, $\frac{1}{8}$ in. and in. $\frac{1}{4}$ apochromatics.



Fig. 1

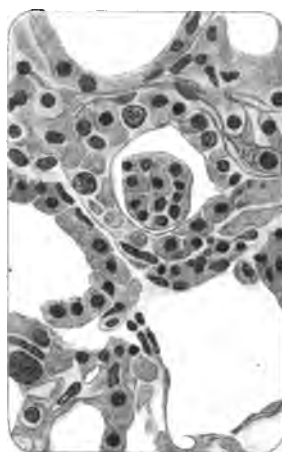


Fig. 2

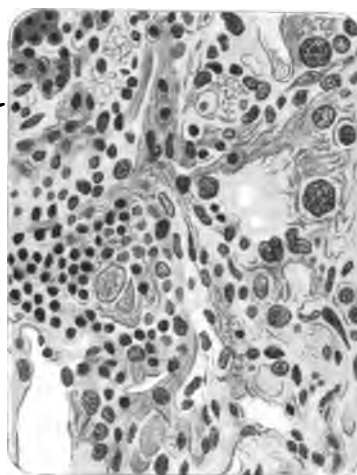
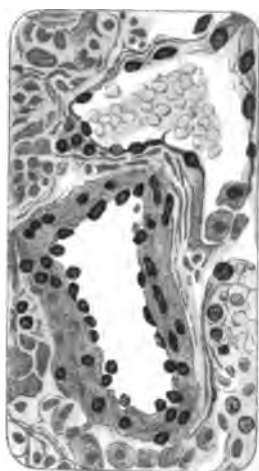
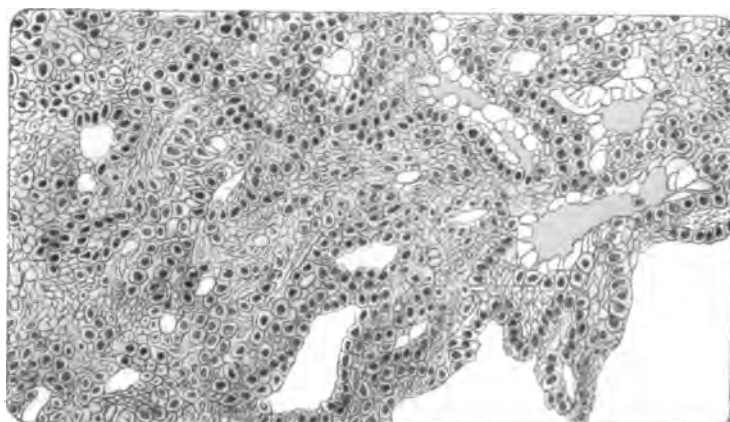


Fig. 3



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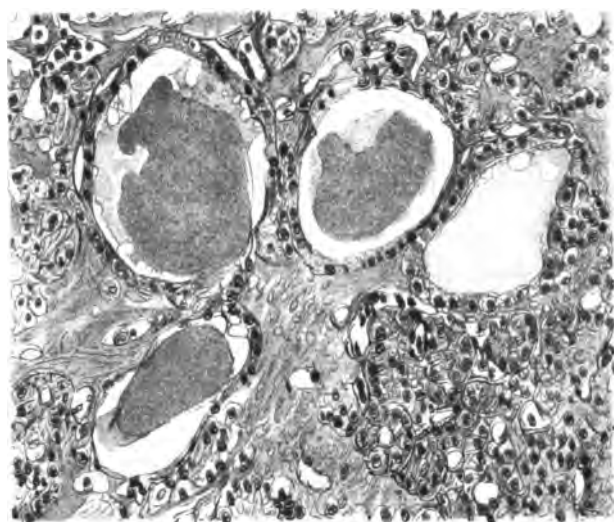


Fig. 1

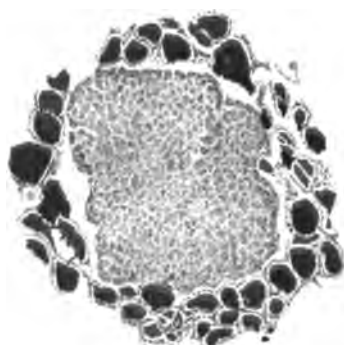


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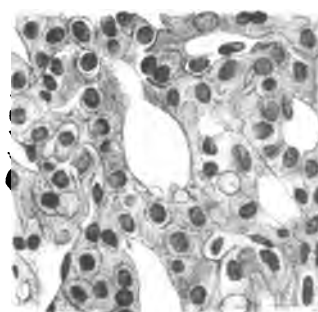


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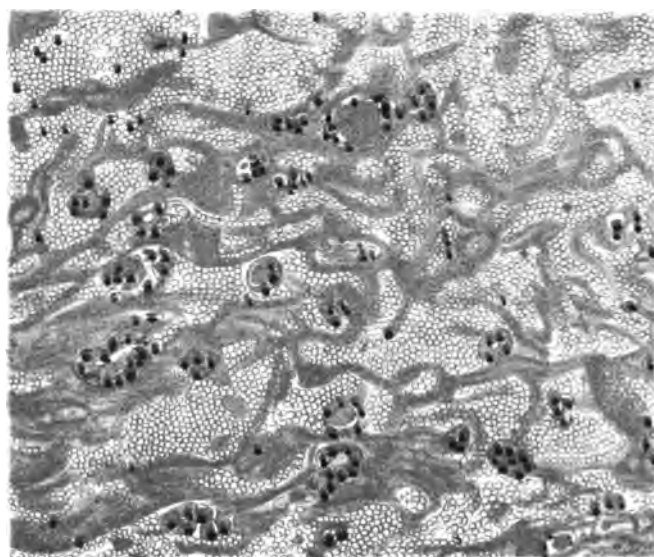


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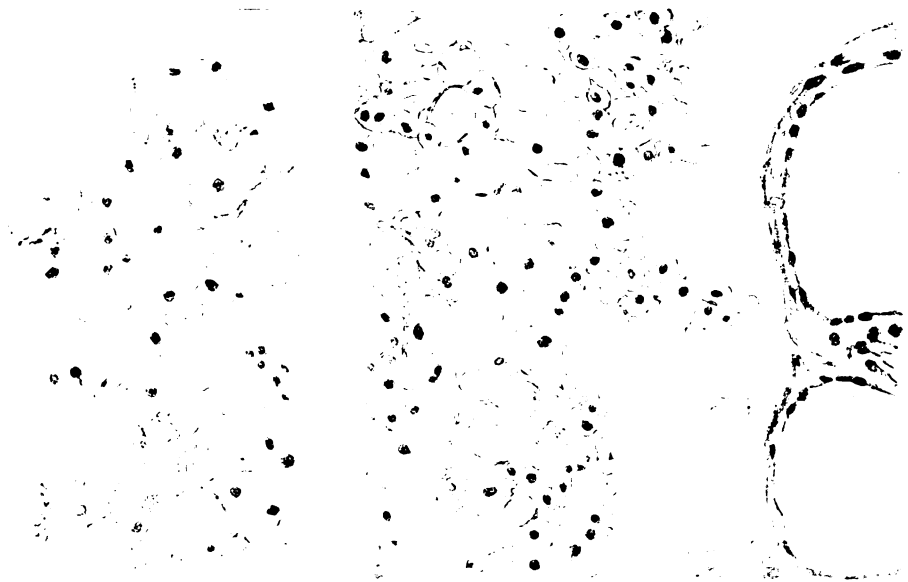


Fig. 10

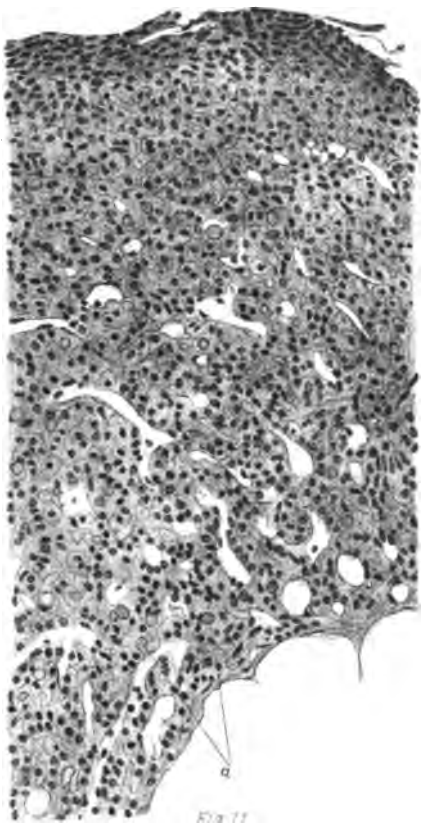


Fig. 11

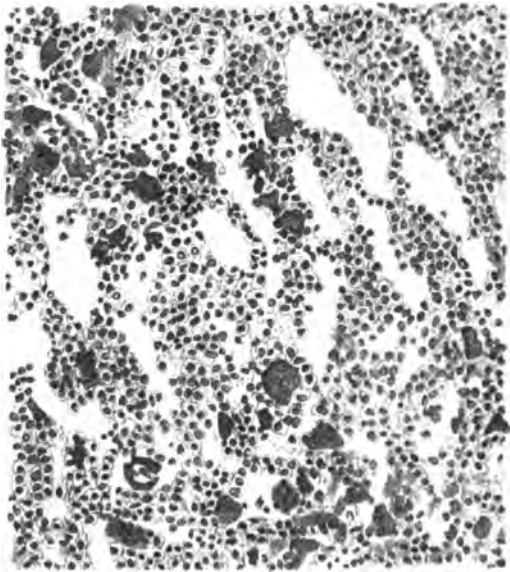


Fig. 12

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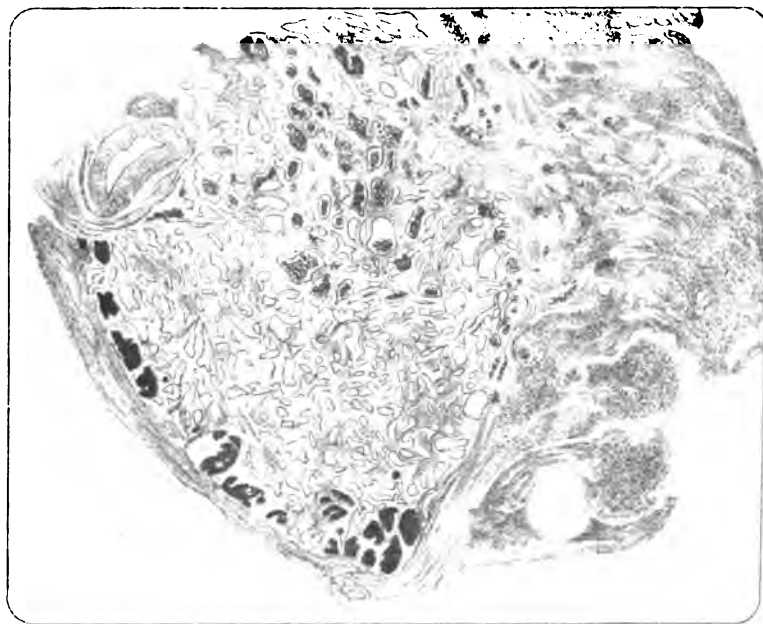


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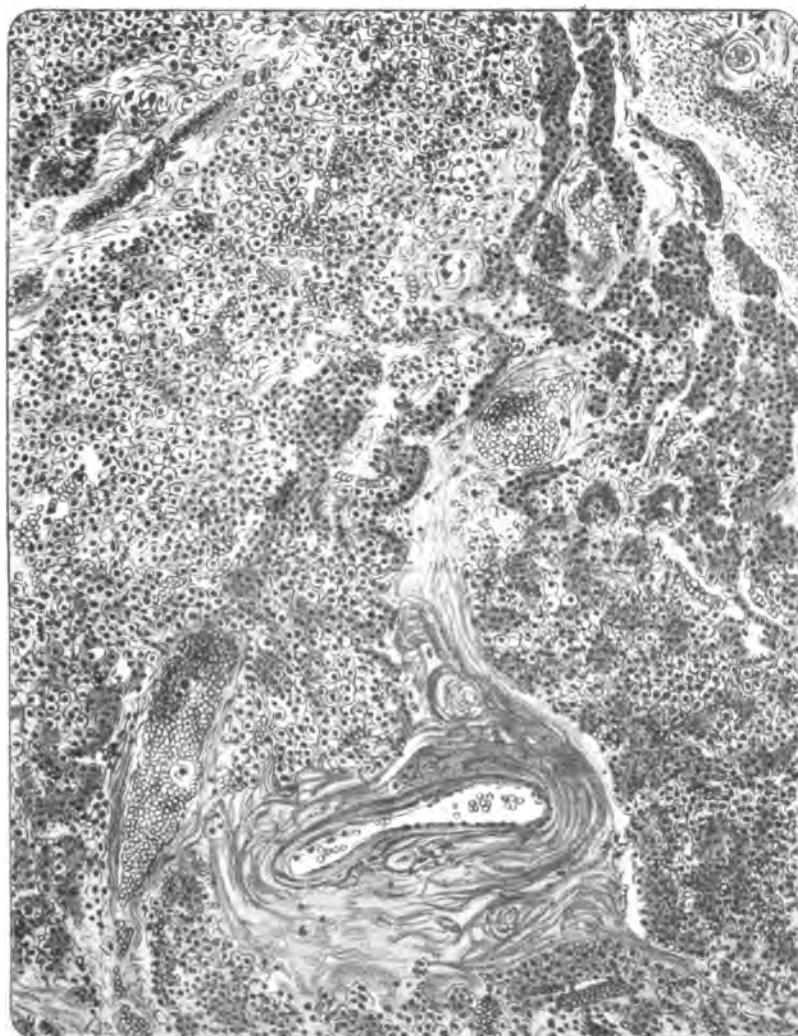


Fig. 2

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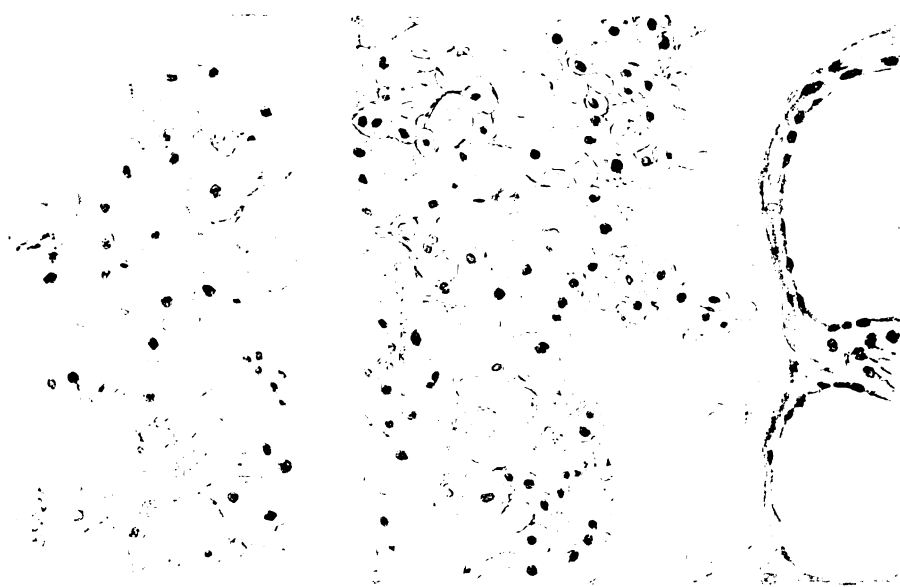


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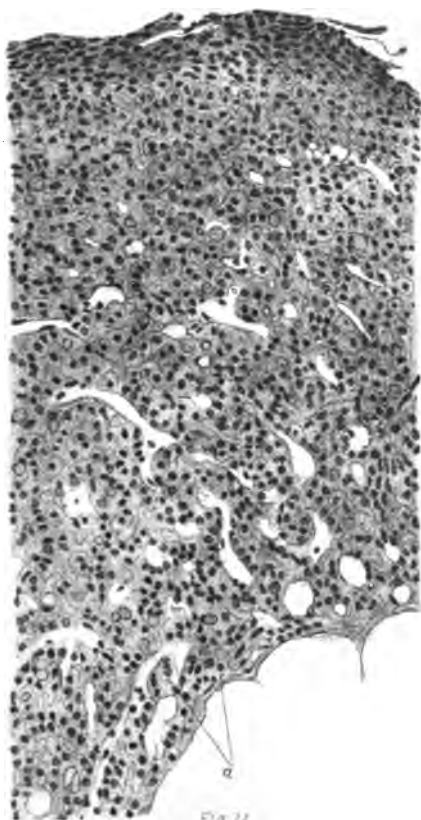


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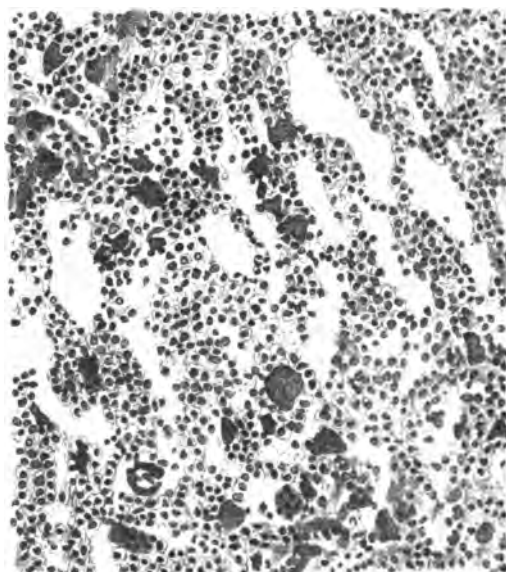


Fig 12

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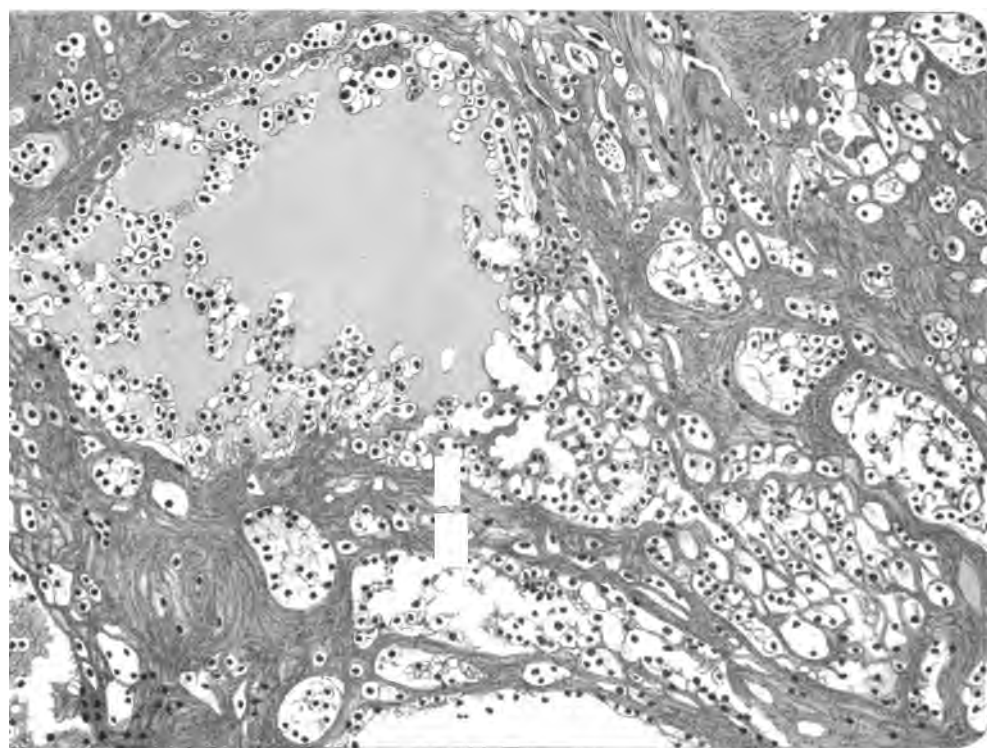


Fig. 13

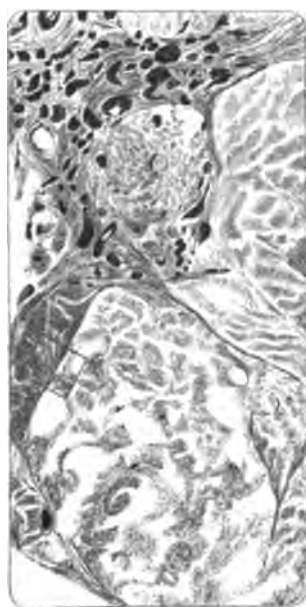


Fig. 14

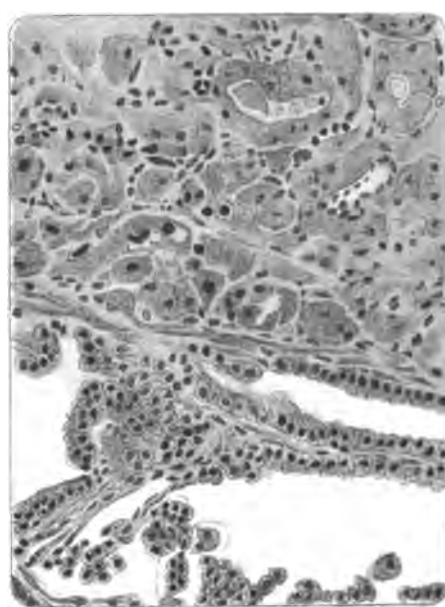


Fig. 15

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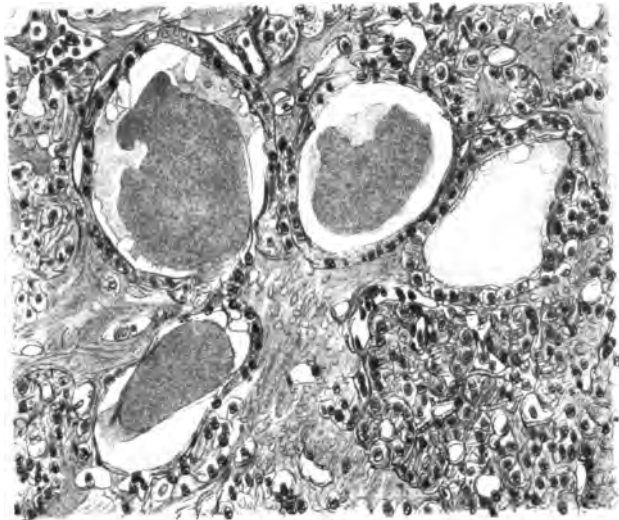


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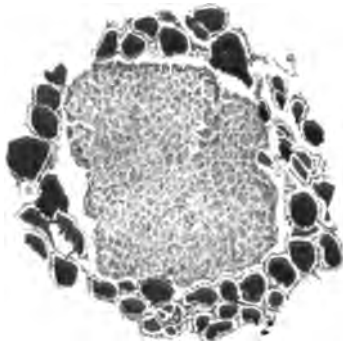


Fig. 2

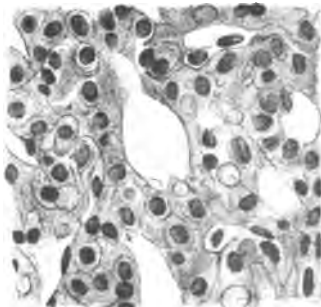


Fig. 3

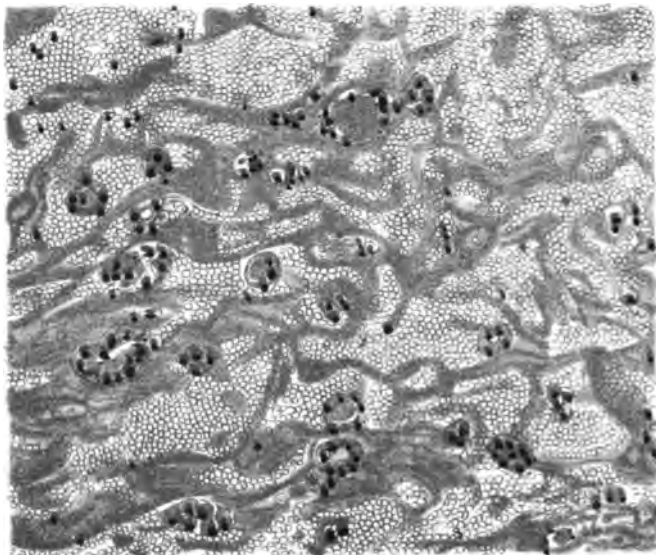


Fig. 4

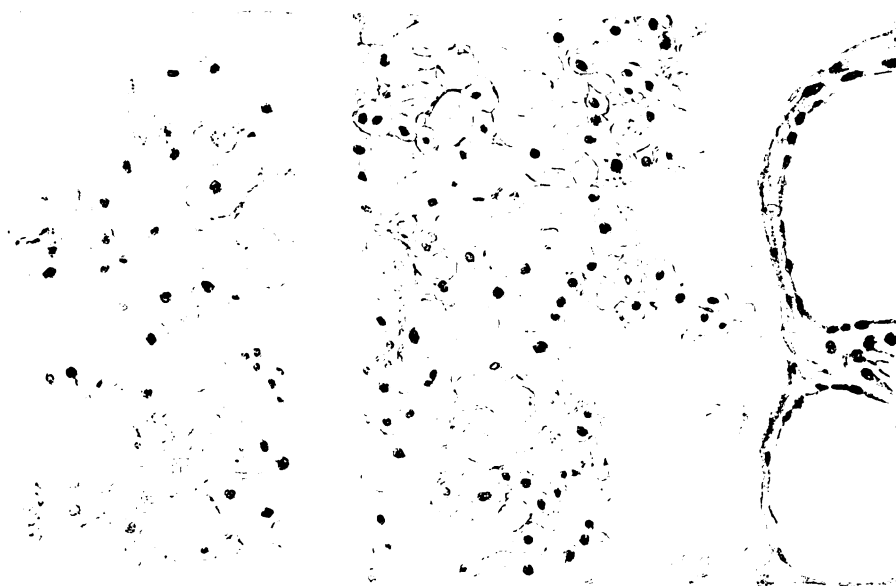


Fig 10

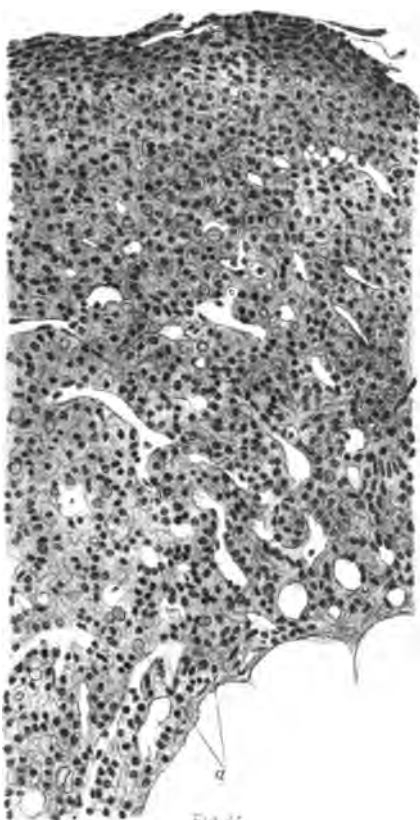


Fig 11

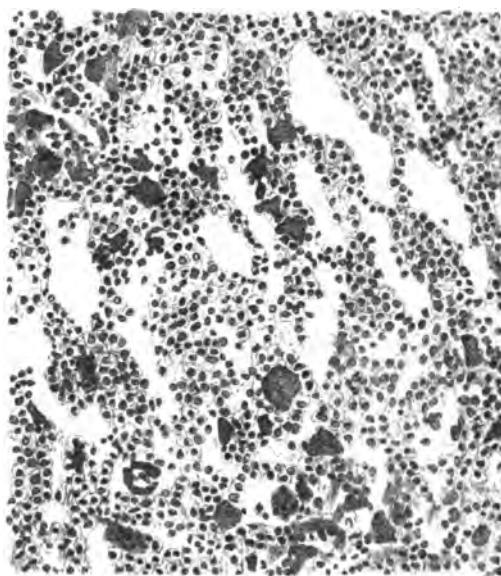


Fig 12

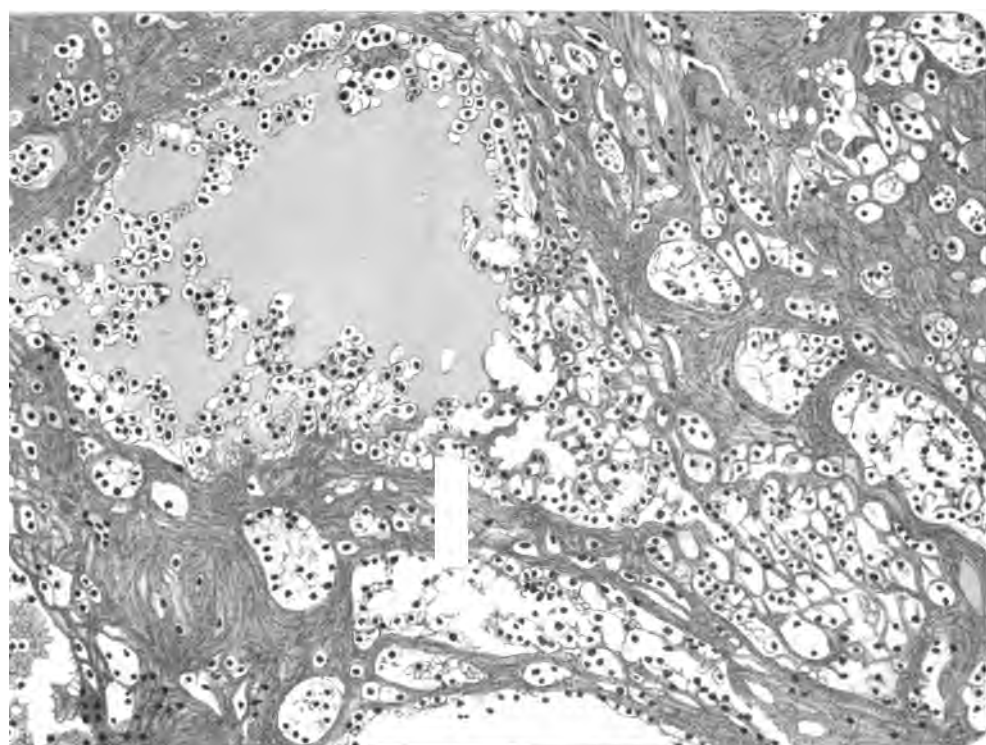


Fig. 13

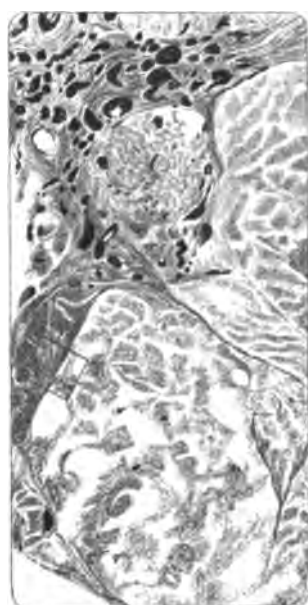
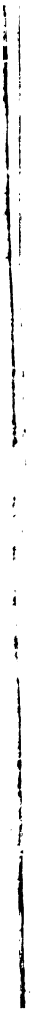


Fig. 14



Fig. 15



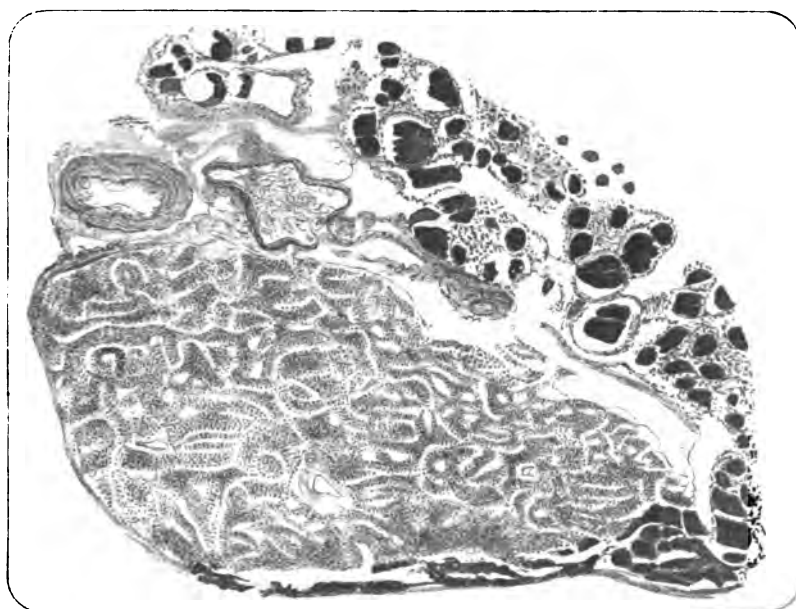


Fig. 16

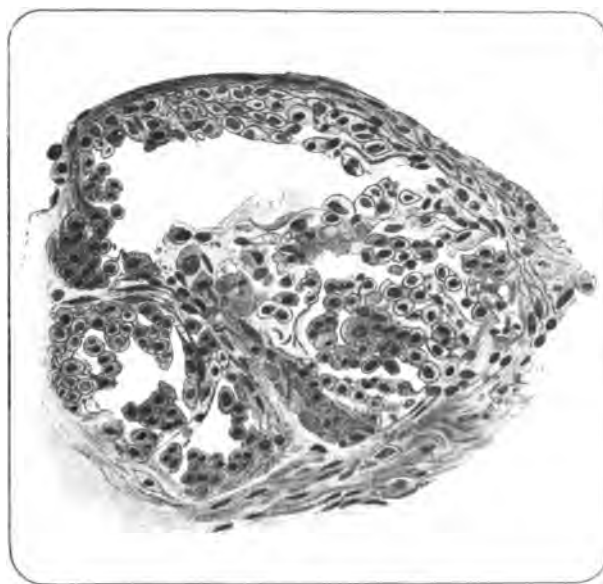


Fig. 17

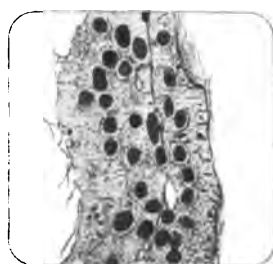


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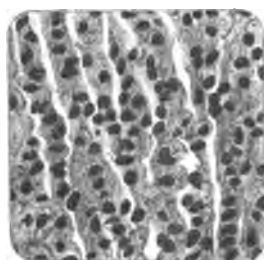


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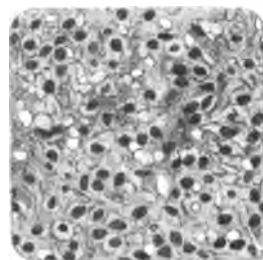


Fig. 20

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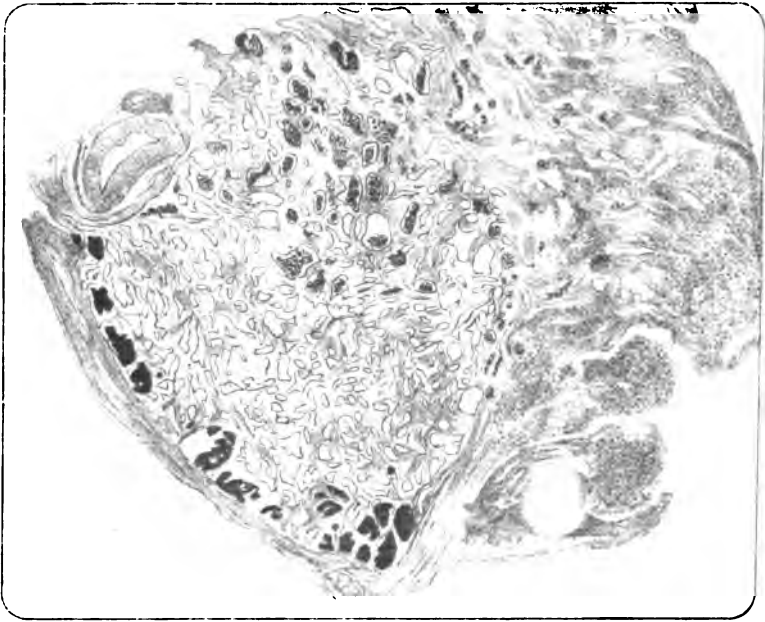


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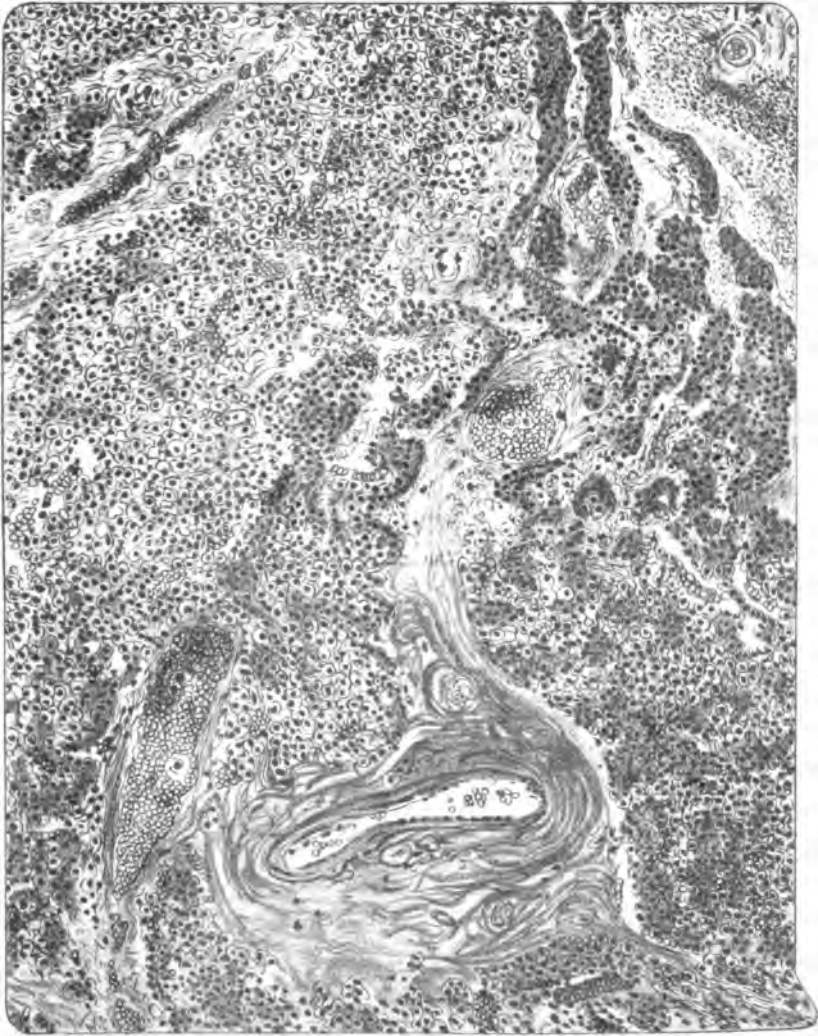


Fig. 2

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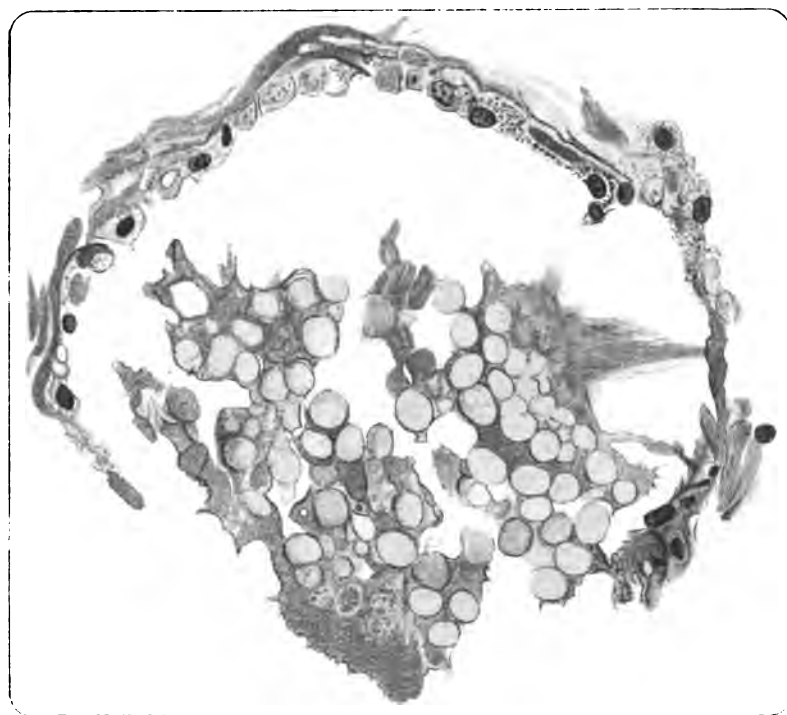


Fig. 24

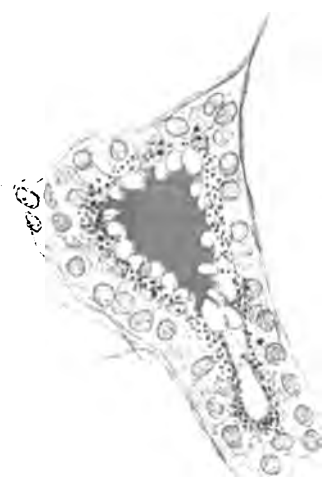


Fig. 25

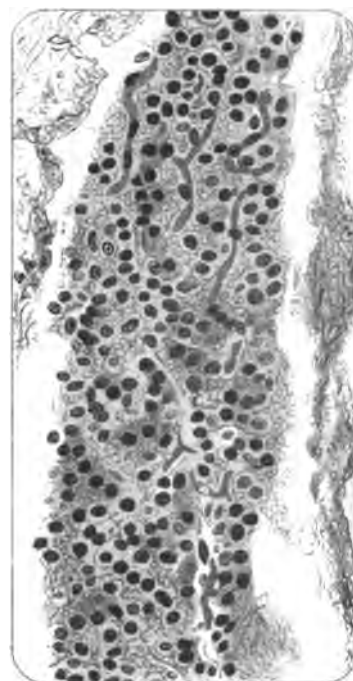


Fig. 26

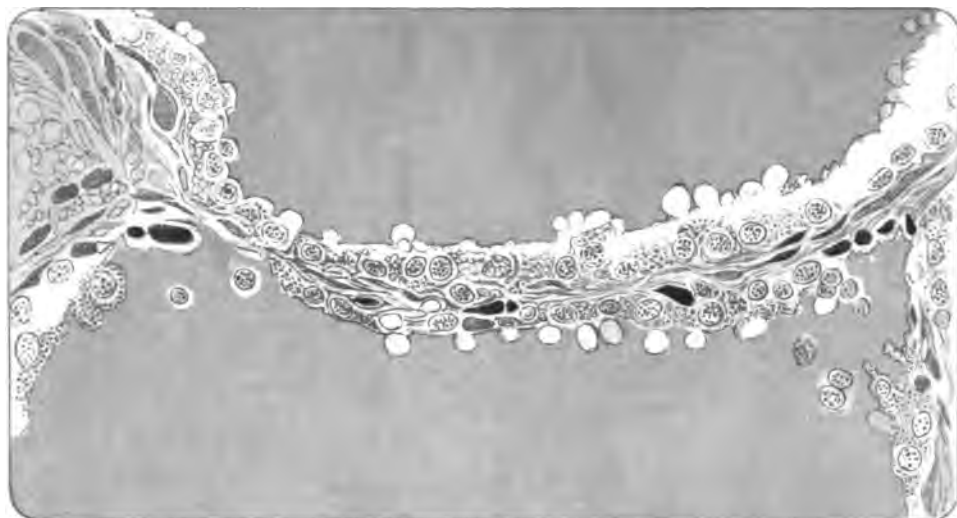


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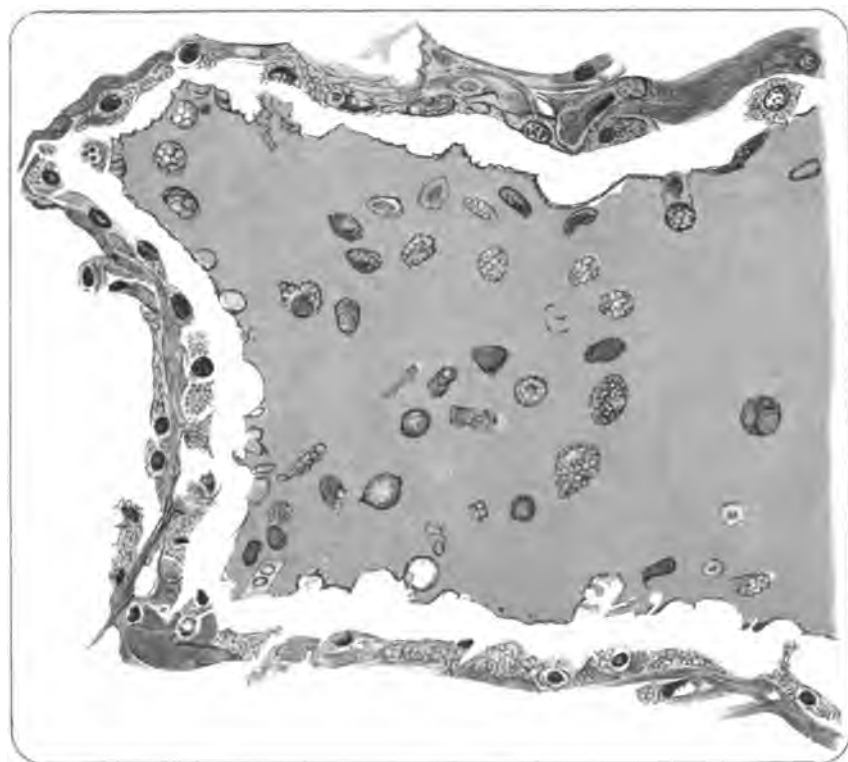


Fig. 2.

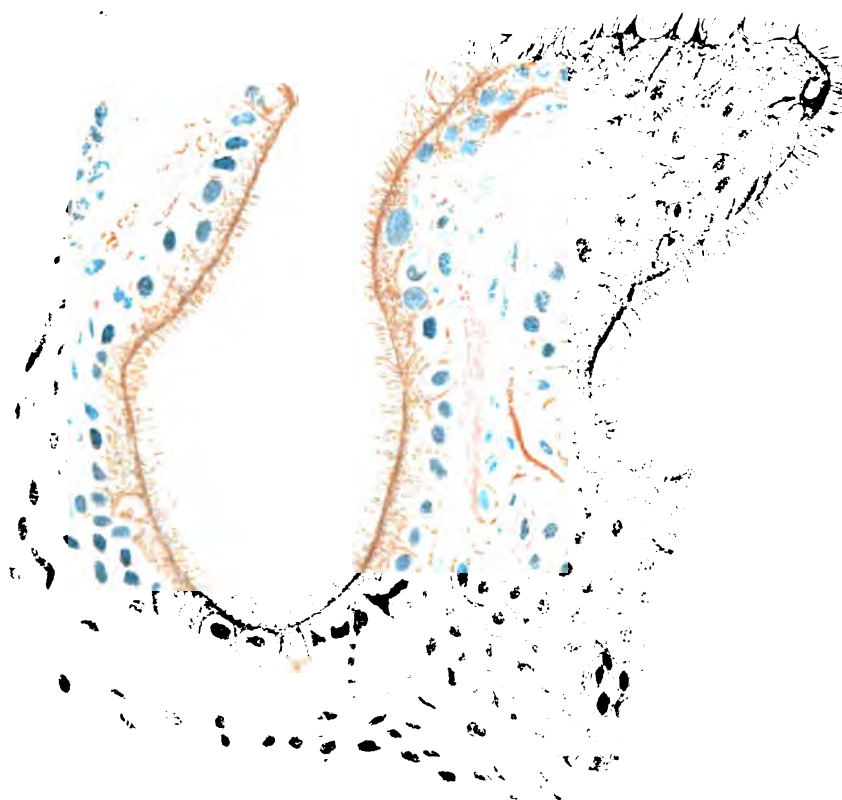


Fig 29

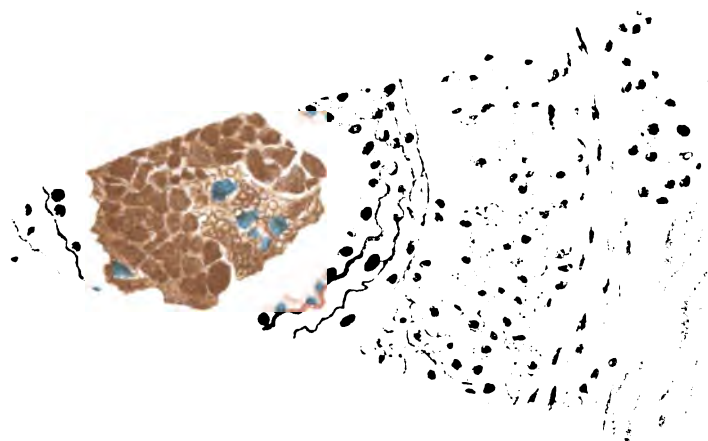


Fig 30

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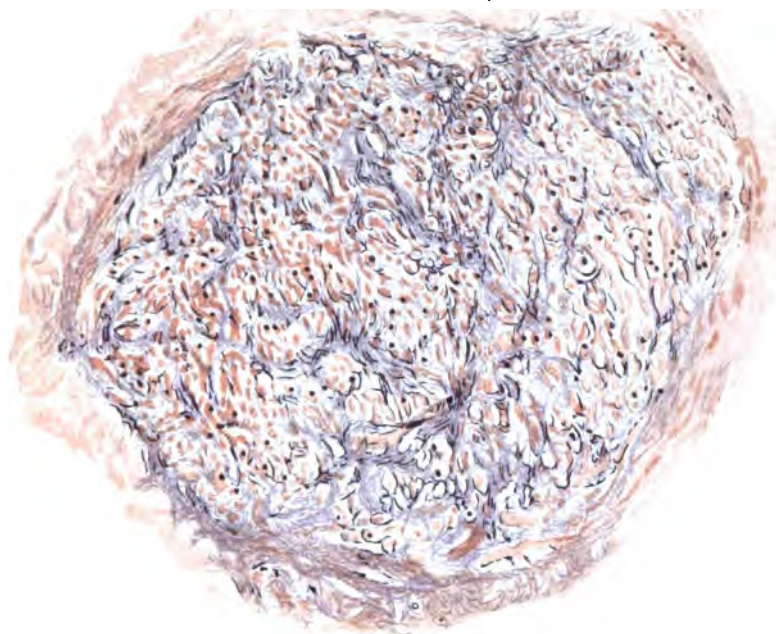


Fig 31

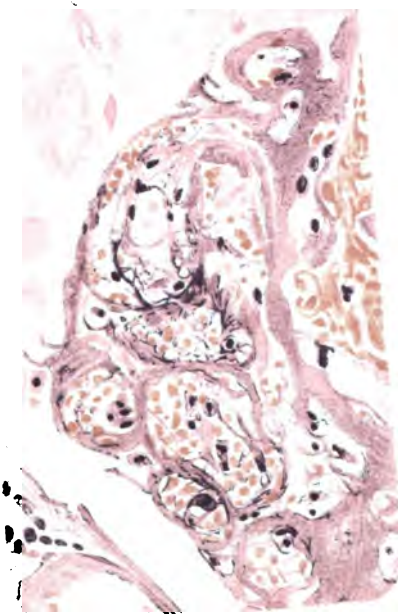


Fig 32

REPORT ON A TUMOUR OF THE SUPRARENAL MEDULLA.

By F. W. EURICH, M.B., C.M. (Edin.), *Pathologist, Lancashire County
Asylum, Whittingham.*

TUMOURS in the region of the kidney and suprarenal always commanding some interest, an account of such a neoplasm may be worth communicating; not only because of its peculiar situation and structure, but also from its possible bearing upon the origin of some forms of renal growths.

The tumour we are about to describe was in no wise instrumental in causing death. The patient, a man aged 38, was in the last stage of general paralysis, death ensuing from that disease and catarrhal pneumonia. With the exception of these and some slight hepatic atrophy no other morbid appearances were found until the right kidney was laid bare, when the following condition was noted. Kidney and suprarenal body were separated from each other to the distance of a couple of inches. While the latter occupied its usual place, there was a slight but distinct downward displacement of the former—insufficient, however, to account for the gap. This was due rather to a congenital atrophy of the kidney, which measured 2 inches in length, $1\frac{1}{2}$ inches in breadth, and $\frac{3}{4}$ inch in thickness. The atrophic organ, as is so frequently the case, presented an overgrowth of fibrous tissue, occupying mainly the papillæ, with a few wedge-shaped patches in the cortex, and compressing the tubules, the epithelium of which was in various stages of degeneration. The capsule, though a little thickened, stripped readily, leaving a smooth cortical surface. The left kidney showed compensatory hypertrophy, but was otherwise healthy. The above-mentioned interval between kidney and suprarenal body was occupied by a tumour, almost spherical, measuring somewhat more than an inch in diameter, of a livid purple, tense to the feel, and encapsulated. Upon its upper pole was perched the suprarenal body, apparently healthy in every respect, and in no wise involved by the tumour mass, from which it was separated by the aforesaid thin capsule. This capsule of fibrous tissue, over the anterior surface of which a few veins were seen to course, was pro-

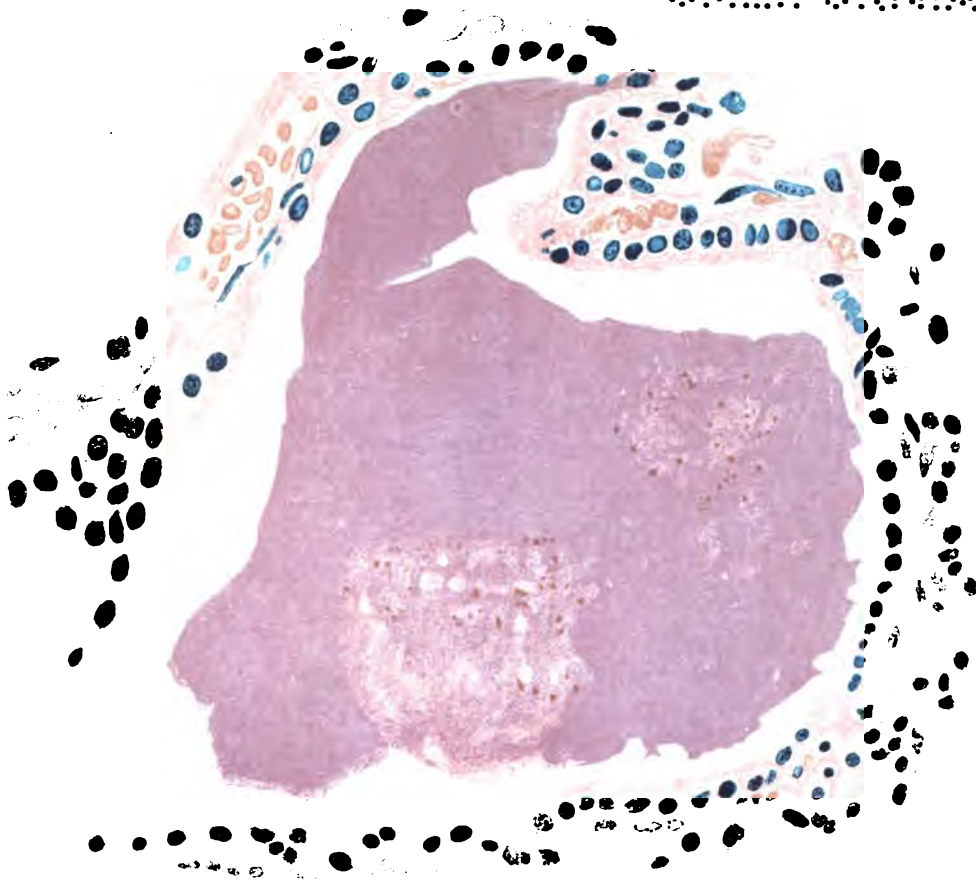


Fig. 33

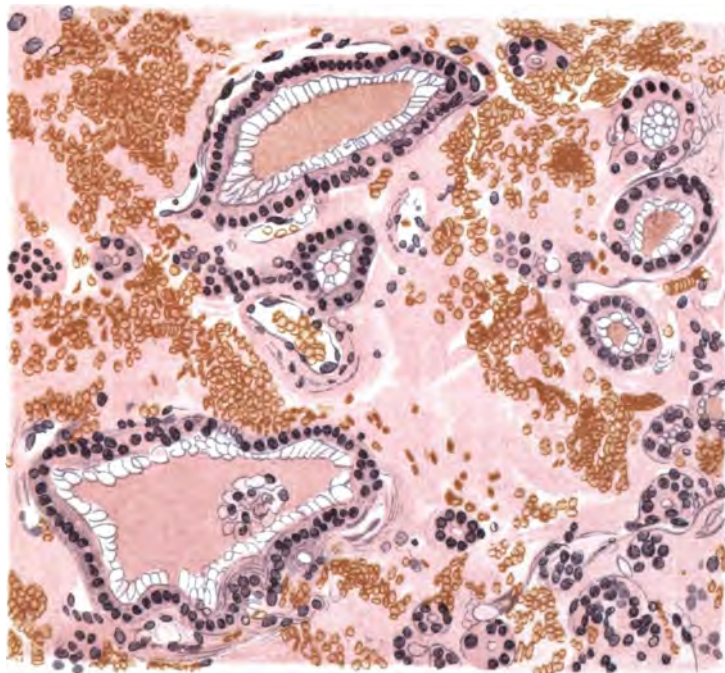


Fig. 34

latter. This is a peculiar homogeneous, hyaline-looking material, lying in the veins and its branches, but apparently not in the larger sinuses. It stains yellowish-brown with picro-carmin, pale red with lithium-carmin, faintly with alum-carmin, and not at all with hæmatoxyline. It does not always quite fill the vessels, but may leave a gap filled with blood corpuscles. Blood cells are also found embedded in the mass itself. Where there is no lining endothelium the tumour cells frequently lie in apparent contact with this substance; and this appearance may, if the vessel be small and if the cells themselves present a yellow tint, closely simulate a giant cell. Prolonged contact with alcohol completely destroys it; and though hyaline-like in appearance, it cannot be considered hyaline in nature. To complete the description we should add that no traces of fat or of glycogen could be detected within the tumour cells by the usual methods.

In structure, therefore, the growth closely resembles that of the medulla of the suprarenal body, though we were unable to convince ourselves of the presence of ganglion cells or nerve fibrils. The absence of all nerve elements may seem, at first, a serious objection to this view. The history of peripheral accumulations of nerve cells meets this objection: they reach their final positions by emigration, and their axis cylinders, as centrifugal outgrowths from the cell bodies, insinuate themselves between tissues and cells, according to laws, as yet not fully understood, till they reach their destination. The nerve cells and fibres within the suprarenal medulla are no exception. It seems to us quite reasonable to suppose that, with the abnormal development and position of this piece of suprarenal medulla, nerve elements may be altogether wanting.

We may now pass to discuss the nature of the growth. For a simple hyperplastic tumour of the medulla the growth is rather large—much larger than is usual in such cases; and is, moreover, separated from the suprarenal body by a thin capsule of fibrous tissue. Yet the tumour is, undoubtedly, of the nature of the suprarenal medulla; if the arrangement of cells and vessels were not sufficient evidence, the peculiar substance within the veins would be. Manasse had already noted it, as we subsequently found, in the suprarenals of adult lower mammalia; we ourselves have lately seen it with Golgi's "rapid method" in the suprarenal of a foetal calf at the fifth month, and also in that of a new-born kitten; but we have been unable to find in the literature at our disposal any previous reference to it in the case of the human being. The idea that it is an artefact can scarcely be entertained; the supposition that it may prove to be a natural secretion seems to us the more probable. We content ourselves, however, with the mere description of it, feeling that a discussion as to its possible function would, with present data, be premature.

Of more immediate interest is the question as to the relation, if

FIG. 5.—Section of goitre in a case of Graves's disease. The great amount of secreting cell tissues between the vesicles is to be noticed. ($\times 220$.)

PLATE XXV.

FIG. 6.—Section of goitre in a case of Graves's disease. There is a large amount of young thyroid tissue on the right, below; it will also be seen that the contents of the vesicles have not stained uniformly; there is a central darker (normal) portion and a lighter external layer. ($\times 220$.)

FIG. 7.—Parathyroid of a monkey. The normal thyroid tissue completely surrounds it. It resembles that of the dog, and is devoid of vesicles and of colloid secretion. ($\times 50$.)

FIG. 8.—Represents the thyroid of a sheep. It is a portion of Fig. 11 more highly magnified. The lightly shaded small bodies with their outlines are drops of secretion which have not stained darkly as the colloid in thyroid vesicles does. ($\times 500$.)

FIG. 9.—From an adenomatous goitre. The structure resembles erectile tissue. The spaces containing blood appear to communicate freely. ($\times 200$.)

PLATE XXVI.

FIG. 10.—From the goitre in a case of Graves's disease. The invasion of the colloid substance by large vesicular cells staining with eosin; these cells are the descendants of the secreting cells lining the vesicle; on the left is seen a cell with two nuclei, presumably about to divide. ($\times 380$.)

FIG. 11.—Parathyroid of sheep. The smaller and shaded spaces consisted of drops of secretion, but it does not stain in the same way as the colloid in the vesicles of thyroid tissue proper; the former stains palely, the latter darkly. Fig. 8 shows a portion of this section more highly magnified. ($\times 220$.)

FIG. 12.—From a goitre occurring in a boy. A portion of the tumour was removed by operation on account of pressure symptoms. The structure is mainly cellular; the colloid is recognised by the dark colour it has stained; it is not contained in well-defined vesicles. ($\times 250$.)

PLATE XXVII.

FIG. 13.—From an adenomatous goitre. It will be seen that in the large vesicle occupying the field that the epithelial secreting cells have multiplied and invaded the colloid; to the right, and below, the colloid has wholly disappeared, and here it is difficult to determine the exact limits of the vesicle. ($\times 220$.)

FIG. 14.—From a fatal case of Graves's disease. Above, and to the left, the colloid is recognised by its dark staining. Above, and to the right, the tissue is papillomatous in character; the portion marked with a square is shown more highly magnified in Fig. 15. ($\times 25$.)

FIG. 15.—The part marked in Fig. 14 more highly magnified. ($\times 225$.)

PLATE XXVIII.

FIG. 16.—The parathyroid of a dog. Above, and to the right, thyroid proper tissue is seen, with the colloid darkly stained: it will be noticed that in the parathyroid there are no vesicles and no colloid; that is to say, no secretion that stains in the same way that the colloid does. ($\times 50$.)

FIG. 17.—Section of the thyroid of a cretin. It was obtained from the body of a girl aged 10 years, who was the subject of cretinism. The thyroid did not appear atrophied. The sections show but little colloid in the vesicles, and there is a considerable multiplication of the secreting cells lining them.

such tissues as the neighbouring muscles and suprarenal corpuscles, may also furnish "residues." Rhabdomyomata and tumours, with the characters of the suprarenal cortex, have already been differentiated; but future investigations must show whether from the remaining mass of renal tumours there can be separated such as bear the stamp of medullary origin.

A SIMPLE AND RAPID METHOD OF DESICCATING SERUM AND KEEPING IT STERILE DURING THE PROCESS.

By CHARLES MARTIN, M.B., B.Sc., *Demonstrator of Physiology in the University of Sydney.*

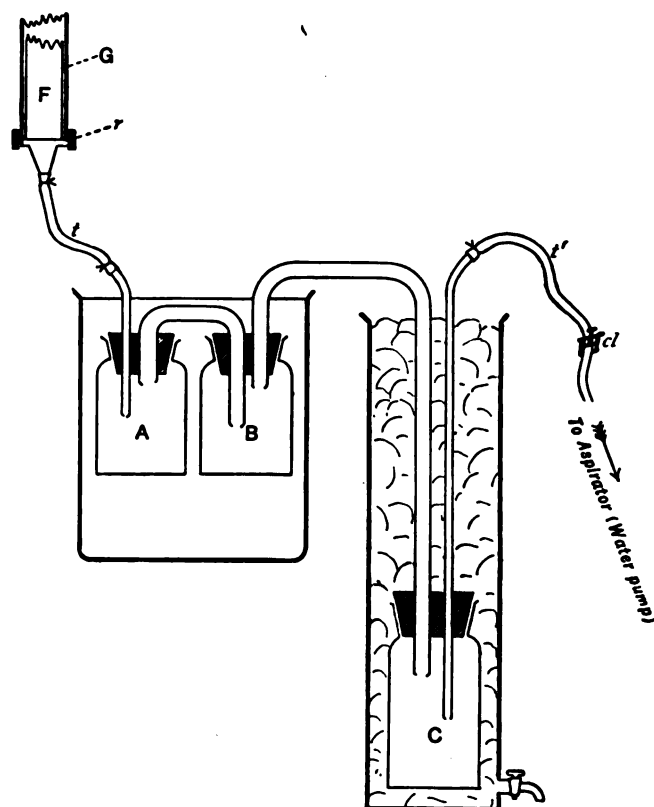
THE following description of a simple and efficacious method of obtaining dried *sterile* serum may be of service to those who, like myself, have wanted to desiccate about a litre at one time, and to obviate any possibility of contamination during the process.

After a few preliminary trials, I found that some form of distillation under diminished pressure, by which bubbling occurred, was much the most efficient method. Having come to this conclusion, there seemed no need to perform the filtration through a Pasteur-Chamberland filter, and the desiccation in two separate stages; if they were both performed together, it would be to the great advantage of the latter.

The arrangement of the apparatus is shown in the figure. F represents the filter, surrounded by a piece of glass-tubing of the same external diameter as the flange of the filter, to which it is fixed by a piece of rubber-hose (*r*) encircling them both. A and B are wide-mouthed bottles of about 350 c.c. capacity. A is connected with F by means of a piece of glass tube and a short length of rubber-tubing *t*, and with B by a horseshoe-shaped piece of glass tube of about 1 cm. internal diameter, passing through the rubber-corks in the mouths of the bottles. B and C are connected by a piece of the same tube, the short limb of which passes just through the cork of B, and the long limb through the cork of C. Another long glass tube of smaller bore passes from C, and to the upper end of this 18 ins. of stout rubber tube is attached, on which is a screw clamp (*cl*).

Before using the apparatus, the clamp (*cl*) is screwed up and the whole sterilised. By rotating the tube connecting B and C in its socket in the rubber-cork of B, the apparatus takes up very little room, and can be inserted in the steam-chamber of a Koch's steriliser. When cool, the glass cylinder G is filled with serum, and A and B surrounded by a vessel containing water at 40° C., the temperature of which is maintained constant by a thermo-regulator in connection with

the gas-supply to a small Bunsen burner underneath. C is immersed in a tall jar containing ice. The rubber-tube t' is connected with a water-pump,¹ and the clamp (cl) relaxed. The serum is aspirated into A, and arrives in the form of great bubbles, which present a large surface for evaporation, so that it dries as rapidly as it comes in. If any bubbles escape out of A they are dried in B. The water condenses in C. By this means *the serum can be desiccated as quickly as it will filter, and one obtains it in the dry condition as quickly and with as little trouble as in the fluid state, and it is impossible for it to become contaminated in the meantime.*



When all the serum has been sucked through and dried, if not immediately wanted it may be allowed to remain in the bottles until another supply of serum is ready to be dried. All that is necessary in the meantime is to screw up the clamp (cl), disconnect G and F, and clean the outside of the filter with water and a brush. When the glass cylinder is washed and reconnected with the filter, the apparatus is ready for use again. The serum dries mostly on the sides of the bottle,

¹ A Wolff's bottle must be inserted between the Bunsen's pump and the tube t' , to obviate back-flow of water into the apparatus, in case the pressure in the water-pipes should vary suddenly during the operation.

but when thoroughly dry it contracts away from the glass, and falls in dark amber translucent flakes on the bottom of the bottles, so that no scraping is required. If the serum does not come through one filter with sufficient rapidity, two can be employed by inserting a Y-piece of glass-tube in the rubber-tubing (*t*). With two filters and bottles of the capacity mentioned, I have desiccated a litre of serum at 40° C. in less than 24 hours. Ice is not essential, the tube connecting C with B may be surrounded instead by a Liebig's condenser, but the greater the difference of temperature between C and B the quicker the evaporation.

The advantages of some such method as I have described are (1) that it dries sterile serum under conditions in which it is impossible for contamination to occur; (2) it does not require any special apparatus, but is entirely constructed of materials which are present in every laboratory.

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